THE EFFECT OF CERTAIN AMINO ACIDS ON THE GROWTH OF CERTAIN BACTERIA

by

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THE EFFECT OF CERTAIN AMINO ACIDS ON THE GROWTH OF CERTAIN BACTERIA

History

Pere in 1892 was the first to have done any work in the nitrogen nutritional requirements of bacteria. He noted that a certain bacterium would not grow upon purified egg albumin as the only source of nitrogen but would grow on the same medium to which a dilute solution of HCL had been added. Bainbridge (1911) showed that certain bacteria, among which were B coli communis and B typhosus, did not break down appreciable quantities of purified unaltered egg albumin and serum protein, even in the presence of non-protein nitrogenous food, to ensure vigorous bacterial growth. Sperry and Rettger (1915) corroborated this work showing that purified egg albumin had a greater inhibiting influence than a control medium of inorganic salts. Also they found that the vegetable protein edestin did not undergo disentegration by direct bacterial action. Their interpretation of the results was that in pure protein medium, bacteria are not supplied with nitrogen which is available for their immediate use and so come to standstill or die of inanition. If these media contained peptone, a decomposition product of protein, or pure protein in the presence of a cleavage producing agent, as dilute alkali, acid, or proteolytic enzyme, disentegration followed. That is, the organisms not breaking down the pure protein contained no enzymes which would attack the nitrogen in a stably constructed molecule.

Rettger, Berman, and Sturges (1916) report that non-liquefying bacteria used no nitrogen of purified coagulated egg albumin and liquefying organisms used very little. In absence of utilizable nitrogen the bacteria underwent autolysis. The results of Sturges and Rettger (1922) indicate that proteolytic bacteria of the type of B prodigiosus, B pyocyaneus, and B subtilis autolyzed rapidly. Of the pathogenic cocci studied, gonococcus, menin-gococcus, and pneumococcus - altho less marked, showed evidences of true autolysis.

In continuance with the work of Rettger, Berman, and Sturges, Berman and Rettger (1918) found that coagulated egg albumin was used by the most active proteolytic bacteria only after at least partial disintegration or cleavage by enzymes or other protein destroying agents. Purified proteose was attached by the actively proteolytic forms - B subtilis, B ramosus, and B prodigiosus - in the presence of meat extract. In the absence of meat extract only B prodigiosus could develop. From this they conclude that the meat extract gave the organisms a start and enabled them to elaborate their enzymes.

Further, Robinson and Rettger (1918) stated that a protein free enzyme-digestion product was better for non-pathogenic and pathogenic organisms including pneumococcus, meningococcus, and B pertussis than a peptone medium. They suggest that the bacteria depend for their nitrogenous food supply on the less complex products, such as

amino acids and perhaps some of the simpler polypeptids rather than upon proteins.

The problem then developed into the question of the availability of the nitrogen in the amino acids for bacteria. This phase, however, was not new because it had previously arisen with the need of a medium of definite chemical composition.

Galimard and Lacomme (1908) working qualitatively with amino acids as the sole source of nitrogen, report that glycocoll supported B pyocyaneus, B prodigiosus, B coli R", Friedlander's bacillus, and B subtilis. Leucine supported B pyocyaneus, B coli R", B prodigiosus, and the pneumococcus. Tyrosine supported B pyocyaneus, the bacillus of green diarrhea, B coli R", B paratyphosus B, B psittacose, V cholerae, coccus (Lille). Arginine supported the greatest number of organisms, thirteen of the twenty-four species grew. Lysine and aspartic acid supported the growth of but one single species - B pyocyaneus.

In 1919 Koser and Rettger reported that B pyocyaneus, B paratyphosus B, B paratyphosus A, B sanguinarium, and the cholera vibrio appeared to be able to utilize glycine, leucine, valine, glutamic acid, aspartic acid, lysine, phenylalanine, histidine, tyrosine and tryptophane. With the exception of the choleria vibrio, the organisms which utilized the amino acids could as readily initiate development on diammonium acid phosphate.

Raistrich (1919) found that B paratyphosus A, B para-

typhosus B, B faecalis alcaligenes, and B pyocyaneus could use the nitrogen of the iminazol ring whereas B vulgaris could not. Raistrich and Clark (1920) found that this organism, B vulgaris, was also unable to rupture the indole ring, while B pyocyaneus, B flourescens, and B prodigiosus could. Armand Delille (1913) used glycerin bouillon and the amino acids glycine, alanine, leucine, glutamic and aspartic acids, l-tyrosine, phenylalanine, phenylglycine, cysteine, and asparagin. Their results indicated that none of the amino acids containing an aromatic radical permitted the culture of bacteria. All of the amino acids of the saturated aliphatic series gave a more or less abundant growth; aspartic acid and 2% glycine were especially rich.

B coli was found by Zunz and Gvorgy (1916) to utilize glycine and glycl-tryptophane as the sole source of nitrogen. Whereas Otsuka (1916) reports that glycl-tryptophane and glycyl-tyrosine were broken up by Staphlococcus aureus and B prodigiosus but not by B coli. Mellanby and Twort (1908) stated that a bacillus of the colon group decomposed histidine to p-imidazol-ethylamine in a synthetic medium containing ammonium tartrate. B coli was shown by Sasaki (1914) to utilize tyrosine in a medium containing ammonium carbonate.

Mayer and Schaeffer (1919) contend that the imidazol or guanidine nucleus is necessary for the growth of the tubercle bacillus. Long (1919) noted that alanine in the

presence of glycerol supported the growth of B tuberculosis and on the substitution of phenylalanine for alanine the growth was diminished. The effect may have been due to the phenol radical.

Franzen and Egger (1914) compared the nutritional value of glycine, alanine, and asparagin as the sole source of nitrogen for B prodigiosus. Asparagin was reported the best, then followed alanine with glycine closely behind. Davis and Ferry (1919) in studying the effect of amino acids on B diphtheria noted that with cystine in beef infusion, a strong toxin was elaborated and that the strength of the toxin diminished when the organism was grown on tryptophane, glutamic hydrochloride, sodium asparaginate, and glycocoll. Practically no toxin was produced on leucine, tyrosine, and histidine dichloride, altho the growth was moderate.

Braum and Cohn-Bronner (1921) using qualitative tests found that glycine, l-leucine, and l-tyrosine were unsuited for the growth of B paratyphosus B, while d-alanine, aspartic acid and glutaminic acid gave good growth. L-tryptophane allowed only a feeble growth.

Francis (1923) reports that B tularense does not grow on plain beef infusion but grows with the addition of 0.1% cysteine hydrochloride or cystine. Tryptophane, tyrosine, histidine dichloride, phenylalanine, leucine, lystine dichloride, and glutamic acid hydrochloride were added to plain beef infusion peptone agar in the same proportion (0.1%) in

which cystine had been found favorable but no growth took place when the media were inoculated with B tularense.

B botulinus was studied by Wagner and Meyer (1925) as to its nitrogen utilization. They conclude that B botulinus apparently lacks the ability to synthesize nitrogen groups from single amino acids and ammonium salts.

Burrous and Neymann (1917) have shown that amino acids, glycocoll, alanine, cystine, valine, leucine, phenylalanine, tyrosine, tryptophane, oxyproline and asparagine were toxic in isotonic solutions for tissues of embryonic chicks.

Wyon and McLeod (1923) qualitatively working on the inhibition of bacterial growth in peptone media by amino acids report that the order of potency of amino acids as judged by the molecular concentrations at the inhibitory threshold is as follows: histidine, tyrosine, tryptophane, cystine, leucine, alanine, glycine and glutamic acids. They conclude that the most potent are the cyclic compounds and that the potency of the monoamino mono-carboxylic acids increases with the molecular weight. Of the dicarboxylic acids, glutamic acid is of low potency and aspartic acid showed no inhibition at relatively high concentrations. Gordon (1924) did not find the results of the inhibitory effects of amino acids as marked as Wyon and McLeod. He found, further, that the growth of the anaerobes was probably improved by the presence of cystine.

Treece (1926) showed that tyrosine in 0.1% concentration inhibited the growth of B coli and delayed the product-

ion of H_2S from cystine.

INTRODUCTION

The scope of this problem has been divided into two parts. The first part consists of the qualitative determination of the effect of varying concentrations of alanine, glycine, aspartic acid, phenylalanine, tyrosine, and tryptophane on B prodigiosus, B dysentery Flexner (82), B coli communis (52), B paratyphosus A (Minnesota 948), B paratyphosus B, B aerogenes (61), B aerogenes, B cloacae (71), B tuberculosis (161), B pyocyaneus, and B subtilis. The second part consists of the quantitative determination of the effect of varying concentrations of glycine, aspartic acid, and tryptophane on the growth rates of B coli communis (52), B aerogenes, B paratyphosus B, and B dysentery Flexner (82) in media with and without the addition of 1% Difco Bactopeptone.

PART I

METHODS

The medium used was an adaption of the formula given by Treece (1926). The percentage of dextrose was reduced to eliminate the possibility of a too great increased acidity upon incubation of the culture. In preparing the media, the amino acid in each case was added directly to 100-150cc base medium which was made up in liter quantities containing:

7gr disodium hydrogen phosphate
2gr potassium acid phthalate
1gr dextrose
1000 cc de-ammonized distilled water

In order to permit only the minimum absorption of ammonia and to render the solution alkaline, the medium was adjusted to Ph 8,4.

The 250 cc Erlenmeyer flasks in which the media were sterilized were tightly stoppered, see accompanying figure, with two-hole rubber stoppers, one hole for a short right angled piece of glass tubing and the other for a bent 10cc pippette extending down below the surface of the liquid in the flask. Before autoclaving the pippette was drawn up so that the lower end was above the

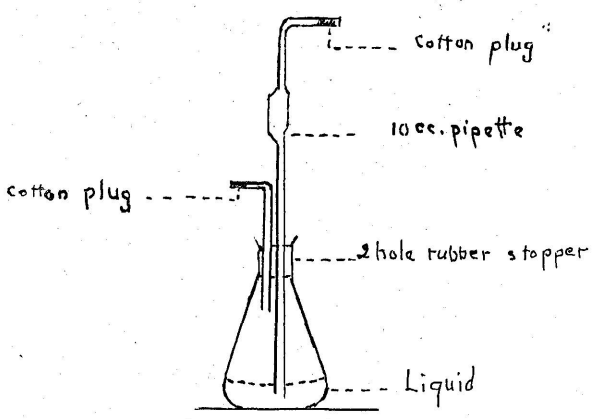


Fig. I

surface of the liquid. Cotton plugs were placed in the upper ends of the glass tubes.

After sterilization the ammonia was removed by a modification of the Folin-McCallum (1912) modification of the Folin air current method. Air freed from ammonia by passage through a wash bottle containing 25% H_2SO_4 was led through the bent 10cc pippette, see the accompanying figure, and bubbled through the medium for 35-45 minutes. The media were then adjusted to PH 6.9, tubed in 5cc portions in sterile chemically clean tubes, and incubated 24 hrs. at 37°C. Contaminated tubes were thrown out. The tubes were always placed in air-tight tin boxes when incubated.

The tubed media was inoculated in duplicate. Base medium controls and negative controls consisting of uninoculated media were run. After 24 hrs. and 7-9 days, plain agar slants were inoculated from the tubes. From the slants on which growth appeared, Gram stains were made. The PH of the media was also determined after 7-9 days.

The concentrations of glycine used were 0.01%, 0.05%, and 1.5%, of alanine, 0.01% and 1.5%. Altho the solubility of aspartic acid is 0.39grs per 100 cc water at 10°C, the increased temperature on autoclaving was found to permit a 0.5% solution in the basic media. Hence for aspartic acid, the concentration 0.5% and 0.05% were used. Phenylalanine and tyrosine were run in the concentration of 0.05%. Tryptophane was limited to the concentrations of 0.05% and 0.75%.

RESULTS

The qualitative growth of the following organisms, B prodigiosus, B dysentery Flexner (82), B coli communis (52), B paratyphosus A (Minnesota 948), B paratyphosus B, B aerogenes, B aerogenes (61), B cloacae (71), B tuberculosis (161), B pyocyaneus, and B subtilis, is recorded in the subsequent tables I, II, III, IV, V, VI, and VII.

Table I. Growth of Various Bacteria in Alanine.

Organism	.010% P_H 6.9					1.500% P_H 6.8				
	Rdgs		Gram		P_H	Rdgs		Gram		P_H
	24 hrs	72 hrs	24 hrs	14 days	14 dd.	24 hrs	72 hrs	24 hrs	9 days	9 days
B. aerogenes	+	+	- Short bac	-Sh. bac.	6.9	±	+	- Short bac	-Short bac	7.0
	+	+	-Short bac	-Sh. bac.	6.9	±	+	-Sh. bac	-Sh bac	7.0
B. prodigiosus	-	-	—	—	6.9	-	±	-Sh. bac	-Sh bac	6.9
	-	-	—	—	6.9	-	±	-Sh bac	-Sh bac.	6.9
B. dysentery Flexner	-	-	—	—	6.9	-	-	—	—	6.8
	-	-	—	—	6.9	-	-	—	—	6.8
B. coli communis	+	+	-Sh bac	- Sh bac	6.9	+	+	-Sh bac	-Sh bac.	6.9
	+	+	-Sh bac	- Sh bac	6.9	+	+	-Sh bac	-Sh bac.	6.9
B. paratyphosus B	-	-	—	—	6.9	-	-	—	—	6.8
	-	-	—	—	6.9	-	-	- rod	—	6.8
B. paratyphosus A	-	-	—	—	6.9	-	-	-Sh rod	—	6.8
	-	-	—	—	6.9	-	-	-Sh rod	—	6.8
B. aerogenes	+	+	- Sh. bac.	-Sh. bac.	6.9	+	+	-Sh bac	-Sh bac	6.9
	+	+	-Sh. bac.	-Sh. bac.	6.9	+	+	-Sh bac	-Sh bac	6.9
B. cloacae	+	+	-Sh. bac.	-Sh. bac.	6.9	±	±	-Sh bac	-Sh bac	6.9
	+	+	-Sh bac	-Sh. bac	6.9	—	±	-Sh bac	-Sh bac	6.9
B. tuberculosis	-	-		+ 1 bac	6.9	-	-	—	+ long bac.	8.0
	-	-		+ 1 bac	6.9	-	-	—	+ long bac.	8.0
B. pyocyaneus	±	+	-Sh. bac	-Sh. bac	6.9	+	+	-Sh bac.	-Sh. bac	8.0
	±	+	-Sh. bac	-Sh. bac.	6.9	+	+	-Sh bac.	-Sh bac	8.0
B. subtilis			B Colonies		6.9	-	±	± bac	± bac	6.8
			± bac		6.9	-	±	± bac	± bac	6.8
Control (neg.)	-	-	—	—	6.9	-	-	—	—	6.8
	-	-	—	—	6.9	-	-	—	—	6.8

Table II. Growths of Certain Bacteria in Glycine

Organism	.05% PH 6.9						.010% PH 6.8							.050% PH 6.9							1.500% PH 6.8						
	Readings				Gram	PH	Readings				Gram		PH	Readings				Gram		PH	Readings				Gram		PH
	24 hrs		72 hrs		7 days	7 days	24 hrs		72 hrs		24 hrs	7 days	7 days	24 hrs		72 hrs		24 hrs	7 days	7 days	24 hrs		72 hrs		24 hrs	7 days	7 days
B. Aerogenes	+		+		-short bacillus	7.0	+	+	+	+	-short bac -short bac	-sh. bac -short bac	6.8 6.8	+		+		-short bac	-short bac	7.0	+	+	+	+	-short bac -short bac	-short bac -short bac	7.2 7.0
B. Prodigiosus	−	−	+	+	-short bacillus -short bacillus	6.6	6.6	+	±	+	+	-short bac -short bac	-short bac -short bac	6.8 6.8	+	+	+	+	-short bac -short bac	-short bac -short bac	6.8 6.8	+	+	+	+	-short bac -short bac	-short bac -short bac	6.9 6.9
B. Dysentery Flexner	−	−	−	−	— —	6.9	6.9	−	−	−	−	— —	— —	6.8 6.8	−	−	−	−	— —	— —	6.9 6.9	−	−	−	−	— —	— —	6.8 6.8
B. Coli Communis	+		+		-short bacillus	6.6		+	+	+	+	-short bac -short bac	-short bac -short bac	6.8 6.8	+		+		-short bac	-short bac	6.8	+	+	+	+	-short bac -short bac	-short bac -short bac	6.9 6.9
B. Paratyphosus B	−	−	−	−	— —	6.9	6.9	−	−	−	−	— —	— —	6.8 6.8	−	−	+ cont	+ cont	— —	± bac ± bac	6.8 6.8	−	−	−	−	— —	— —	6.8 6.8
B. Paratyphosus A	−	−	−	−	— —	6.9	6.9	−	−	−	−	— —	— —	6.8 6.8	−	−	−	−	— —	— —	6.9 6.9	−	−	−	−	cont -sh bac	cont -sh. bac.	6.8 6.8
B. Aerogenes	+	+	+	+	-short bacillus	6.9	6.9	+	+	+	+	-short bac -short bac	-short bac -short bac		+	+	+	+	-short bac -short bac	-short bac -short bac	7.0 7.0	+	+	+	+	-short bac -short bac	-short bac -short bac	6.9 6.9
B. Cloacae	+	+	+	+	-short bacillus	7.0	6.9	−	−	−	−	— —	— contam.	6.8 6.8	+	+	+	+	-short bac -short bac	-short bac -short bac	7.0 7.0	+	+	+	+	-short bac -short bac	-short bac -short bac	
B. Tuberculosis	−	−	+	+	+ bacillus + bacillus	6.9	6.9	−	−	+	+	+ bac −	+ bac + bac.	6.8 6.8	±		+			+ bac	6.8	−	−	−	−	— —	— —	6.8 6.8
B. Pyocyaneus	+	+	+	+	-short bacillus -short bacillus	6.8	6.8	+	+	+	+	-short bac -short bac	-short bac -short bac	6.8 6.8	+	+	+	+	-short bac short bac	-short bac -short bac	7.2 7.2	+	+	+	+	-short bac -short bac	-short bac -short bac	
B. Subtilis	+	+	+	+	± bacillus ± bacillus	6.8	6.8	±	±	+	+	± bac ± bac	± bac ± bac	6.8 6.8	+	+	+	+	± bac ± bac	± bac ± bac	7.0 6.8	−	−	±	±		± bac ± bac	6.8 6.8
B. Dysentery Shiga																											
Control (neg)	−	−	−	−	−	6.9	6.9	−	−	−	−	— —	— —	6.8 6.8	−	−	−	−	— —	— —	6.9 6.9	−	−	−	−	— —	— —	6.8 6.8

Table III. Growth of Certain Bacteria in Aspartic Acid

Organism	0.05% Aspartic Acid P_H 7.0							0.50% Aspartic Acid P_H 6.8						
	Readings		Growth on Slants		Gram Stain		Final	Readings		Growth on Slants		Gram Stain		Final
	24hrs	72hr	24hrs	7days	24 hrs	7days	P_H	24hr	72 hr	24hrs	7days	24 hrs	7days	PH
B. Aerogenes	+	+	Lux.	Lux.	-short bac	-short bac	7.7	+	+	Lux	Lux.	-short bac	-short bac	8.2
	+	+	Lux	Lux	-short bac	-short bac	7.7	+	+	Lux	Lux	-short bac	-short bac	8.2
B. Prodigidsus	+	+	Lux	Lux	-short bac	-short bac	7.7	+	+	Lux	Lux	-short bac	-short bac	8.2
	±	+	Lux	Lux	-short bac	-short bac	7.7	+	+	Lux	Lux	-short bac	-short bac	8.2
B. Dysentery Flexner	-	-	-	2wks -	—	—	7.0	-	-	—	—	—	—	6.8
	-	-	-	2wks -	—	—	7.0	-	-	—	—	—	—	6.8
B. Coli Communis	+	+	Lux.	Lux.	-short bac	-short bac	7.0	+	+	Lux	Lux	-short bac	-sh. bac.	8.0
	+	+	Lux	Lux	-short bac	-short bac	7.0	+	+	Lux	Lux	-short bac	-sh. bac.	8.0
B. ParatyphosusB	-	-	-	-	—	—	7.0	+	+	Meagre	Meagre	-short bac	-sh bac	6.9
	-	-	-	Meagre	—	+ bac	7.0	±	+	Meagre	Lux	-short bac + bac	-short bac + bac	8.2
B. ParatyphosusA	-	-	-	2wks -	—	—	7.0	±	±	Meagre	—	-short bac	—	6.8
	-	-	-	2wks -	—	—	7.0	±	±	Meagre	—	-short bac	—	6.8
B. Aerogenes	±	+	Lux.	Meagre	-short bac	-short bac	7.7	+	+	Lux	Lux	-short bac	-short bac	8.2
	-	-	-	-	—	—	7.0	+	+	Lux	Lux	-short bac	-short bac	8.2
B. Cloacae	-	-	-	2wks -	—	—	7.0	+	+	Mod.	Mod.	-short bac	-short bac	8.2
	-	-	-	2wks -	—	—	7.0	+	+	Mod	Mod.	-short bac	-short bac	8.2
B. Tuberculosis	-	±	Meagre	Mod	+ long bac		7.0	-	+	—	Mod	—	+ long bac	7.0
	-	±	Meagre	Mod	+ long bac		7.0	-	+	—	Mod	—	+ long bac	7.0
B. Pyocyaneus	+	+	Lux	Lux	-short bac	-short bac	7.7	+	+	Lux	Lux	-short bac	-short bac	8.2
	+	+	Lux	Lux	-short bac	-short bac	7.7	+	+	Lux	Lux	-short bac	-short bac	8.2
B. Subtilis	+	+	Lux	Lux	± bac	± bac	7.0	+	+	Lux	Lux	± bac	± bac	8.2
	+	+	Lux	Lux	± bac	± bac	7.0	+	+	Lux	Lux	± bac	± bac	8.2
Control (neg)	-	-	—	—	—	-	7.0	-	-	—	—	—	—	6.8
	-	-	—	—	—	—	7.0	-	-	—	—	—	—	6.8

Legend:
Lux = Luxuriant
Mod = Moderate
bac. = bacillus

Table IV. Growth of Certain Bacteria Tryptophane

Organism	0.05% Tryptophane PH 6.8							0.75% Tryptophane PH 6.8						
	Readings		Growth on Slants		Gram Stain		PH	Readings		Growth on Slants		Gram Stain		Final
	24hr	72hr	24 hrs	7days	24 hrs	7days	Final	24hr	72hr	24 hrs	7days	24 hrs	7days	PH
B. Aerogenes	+	+	Lux	Mod	-Sh. bac	-Sh. bac	6.9	+	+	Lux	Mea	-Sh. bac	-Sh. bac	6.9
	+	+	Lux	Mod	-Sh. bac	-Sh. bac	6.9	+	+	Lux	Mea	-Sh. bac	-Sh. bac	6.9
B. Prodigiosus	+	+	Lux	Mea	-Sh bac	-Sh. bac	6.8	+	+	Lux	—	-Sh. bac	—	6.6
	+	+	Lux	Mea	-Sh. bac	-Sh. bac	6.8	+	+	Lux	—	-Sh. bac	—	6.6
B. Dysentery Flexner	-	±	Mod	—	-Sh. bac	—	6.9	±	-	Mod.	—	-Sh. bac	—	6.8
	-	±	Mod	—	-Sh. bac	—	6.9	±	-	Mod.	—	-Sh. bac	—	6.8
B. Coli Communis	+	+	Lux	Mea	-Sh. bac	-Sh. bac	6.9	+	+	Lux	—	-Sh. bac	—	6.9
	+	+	Lux	Mea	-Sh. bac	-Sh. bac	6.9	+	+	Lux	—	-Sh. bac	—	6.9
B. Paratyphosus B	-	-	Mod	Mea	-Sh. bac	-Sh. bac	6.8	±	+	Mea	—	-Sh. bac	—	6.8
	-	±	Mod	—	-Sh. bac	—	6.8	±	+	Mea	—	-Sh. bac	—	6.6
B. Paratyphosus A	-	±	Mea	Mea	-Sh. bac	-Sh. bac	6.8	+	+	Mea	—	-Sh. bac	—	6.8
	-	±	Mea	—	-Sh. bac	—	6.8	±	+	Mea	—	-Sh bac	—	6.8
B. Aerogenes	+	+	Lux	Mea	-Sh. bac	-Sh. bac		+	+	Lux	Mea	-Sh. bac	-Sh. bac	
	+	+	Lux	Mea	-Sh. bac	-Sh. bac		+	+	Lux	Mea	-Sh. bac	-Sh. bac	
B. Cloacae	±	-	Mod	Mea	-Sh. bac	-Sh. bac	6.8	+	+	Lux	Mea	-Sh. bac	-Sh. bac	6.8
	±	-	Mod	Mea	-Sh. bac	-Sh. bac	6.8	+	+	Lux	Mea	-Sh. bac	-Sh. bac	6.8
B. Tuberculosis	-	+	—	Mea	—	+long bac	6.9	-	±	—	+	—	+long bac	6.9
	-	+	—	Mea	—	+long bac	6.9	-	±	—	+	—	+long bac	6.9
B. Pyocyaneus	+	+	Lux	Lux	-Sh. bac	-Sh. bac		+	+	Lux	Lux	-Sh. bac	-Sh. bac	8.0
	+	+	Lux	Lux	-Sh. bac	-Sh. bac		+	+	Lux	Lux	-Sh. bac	-Sh. bac	8.0
B. Subtilis	±	±	Lux	Lux	± bac	± bac	6.8	±	+	Mod	Mod	± bac		6.8
	-	-	Lux	Mod	± bac	± bac	6.8	±	+	Mod	Mod	± bac		6.8
Control (neg)	-	-	—	—	—	—	6.8	-	-	—	—	—	—	6.8
	-	-	—	—	—	—	6.8	-	-	—	—	—	—	6.8

Legend:
Lux = Luxuriant
Mod = Moderate
Mea = Meagre
Sh. bac = Short bacillus

Table V. Growth of Certain Bacteria in the Base Medium

Organism	Controlling: 1.5% Alanine, 1.5% Glycine, 0.5% Aspartic Acid — P_H 6.8							Controlling: 0.05% Tryptophane, 0.75% Tryptophane — P_H 6.8						
	Reading		Growth on slants		Gram		P_H	Reading		Growth on slants		Gram		P_H
	24hr	72h	24 hr	7days	24 hrs	7days	7days	24h	72h	24 hrs	7days	24 hrs	7 days	Final
B. Aerogenes	−	±	Mod	Mod	-Sh. bac	-Sh. bac	6.8	±	+	Mod	Lux	-Sh. bac	-Sh. bac	6.8
	+	±	Mod	Mod	-Sh. bac	-Sh. bac	6.8	±	+	Mod	Lux	-Sh. bac	-Sh. bac	6.8
B. Prodigiosus	−	+	Mea	Mod	-Sh. bac	-Sh. bac	6.8	−	±	Mod	Mod	-Sh. bac	-Sh. bac	6.8
	−	±	Mea	Mod	-Sh. bac	-Sh. bac	6.8	−	±	Mod	Mod	-Sh. bac	-Sh. bac	6.8
B. Dysentery Flexner	−	−	—	—	—	—	6.8	−	−	Mod	Mod	-Sh. bac	-Sh. bac	6.8
	−	−	—	—	—	—	6.8	−	−	Mod	Mod	-Sh. bac	-Sh. bac	6.8
B. Coli Communis	−	±	Mea	Mod	-Sh. bac	-Sh. bac	6.9	±	±	Mea	Mod	-Sh. bac	-Sh. bac	6.8
	−	±	Mea	Mod	-Sh. bac	-Sh. bac +(+ bac)	6.9	−	±	Mea	Mod	-Sh. bac	-Sh. bac	6.8
B. Paratyphosus B	−	±	Mea	—	-Sh. bac	—	6.8	−	±	Mea	Mod	-Sh. bac	-Sh. bac	6.8
	−	±	Mea	—	-Sh. bac	—	6.8	−	±	Mea	Mod	-Sh. bac	-Sh. bac	6.8
B. Paratyphosus A	−	−	—	—	—	—	6.8	−	−	Mea	Mea	-Sh bac	-Sh. bac	6.8
	−	−	—	—	—	—	6.8	−	−	Mea	Mea	-Sh. bac	-Sh. bac	6.8
B. Aerogenes	−	−	Mea	Mod	-Sh. bac	-Sh. bac	6.8	−	±	Mea	Mod	-Sh. bac	-Sh. bac	6.8
	−	±	Mea	Mod	-Sh. bac	-Sh. bac	6.8	−	±	Mea	Mod	-Sh. bac	-Sh. bac	6.8
B. Cloacae	−	±	Mod	Mod	-Sh. bac	-Sh. bac	6.8	±	±	Mea	Mod cont	-Sh. bac	-Sh. bac +(− bac)	6.8
	−	±	Mod	Mod	-Sh. bac	-Sh. bac	6.8	±	±	Mea	Mod cont	-Sh. bac	-Sh. bac +(− bac)	6.8
B. Tuberculosis	−	−	—	—	—	—	6.8	−	−	—	Mod	—	+ Long bac	6.8
	−	−	—	—	—	—	6.8	−	−	—	Mod	—	Long bac	6.8
B. Pyocyaneus	−	±	—	Mod	—	-Sh. bac	6.8	±	±	Mod	Lux	-Sh. bac	-Sh. bac +(+ bac)	6.8
	−	±		Mod	—	-Sh. bac	6.8	−	±	Mod	Mod	-Sh. bac	-Sh. bac +(+ bac)	6.8
B. Subtilis	−	±	Mea	Mea	± bac	± bac	6.8	−	−	Mea	Mod	± bac	± bac	6.8
	−	±	Mea	Mea	± bac	± bac	6.8	−	−	Mea	Mod	± bac	± bac	6.8
Control (neg)	−	−	—	—	—	—	6.8	−	−	—	—	—	—	6.8
	−	−	—	—	—	—	6.8	−	−	—	—	—	—	6.8

Legend
Lux = Luxuriant
Mod = Moderate
Mea = Meagre
Sh. bac = Short bacillus

Table VI. Growth of Certain Bacteria in 0.05% Tyrosine.

Organisms	Readings		Growth on slants		Gram-stains from slants		P_H 6.8
	24 hr	72 hr	24 hr	7 da.	24 hr	7 da	Final P_H
B. aerogenes	+	+		Lux	-Sh bac	-Sh bac	6.8
	+	+		Lux	-Sh bac	-Sh bac	6.8
B. prodigiosus	–	±	Mea	Lux	-Sh bac	-Sh. bac	6.8
	–	±	Mea	Lux	-Sh bac	-Sh bac	6.8
B. dysentery Flexner	–	–	Mea	—	-Sh bac	—	6.8
	–	–	Mea	—	-Sh bac	—	6.8
B. coli communis	+	+	Lux	Lux	-Sh bac	-Sh bac	6.8
	+	+	Lux	Lux	-Sh bac	-Sh bac	6.8
B. paratyphosus B	–	–	Mea	—	-Sh bac	-Sh bac	6.8
	–	–	—	Cont		Cont	6.8
B. paratyphosus A	–	–	Mea	—	-Sh bac	—	6.8
	–	–	Mea	—	-Sh bac	—	6.8
B. aerogenes	+	+	Lux	Lux	-Sh bac	-Sh bac	6.8
	+	+	Lux	Lux	-Sh bac	-Sh bac	6.8
B. cloacae	–	–	Mea	Mod	-Sh bac	-Sh bac	6.8
	–	±	Mea	Mod	-Sh bac	-Sh bac	6.8
B. tuberculosis	–	–	—	+	—	+ bac	6.8
	–	–	—	+	—	+ bac	6.8
B. pyocyaneus	+	+		Lux	-Sh bac	-Sh bac	6.8
	+	+		Lux	-Sh bac	-Sh bac	6.8
B. subtilis	–	±	Mea	Mea	± bac	± bac	6.8
	–	±	Mea	Mea	± bac	± bac	6.8
Control	–	–	–	–	–	–	6.8
	–	–	–	–	–	–	6.8

Legend:

Mea = Meagre
Lux = Luxuriant
Mod = Moderate
Sh bac = Short bacillus

Table VII. Growth of Certain Bacteria in 0.05% Phenylalanine

Organisms	0.05% Phenylalanine PH 6.8							Base Medium for {0.05% Phenylalanine, 0.05% Tyrosine} PH 6.9						
	Readings		Growth on Slants		Gram Stain		PH Final	Readings		Growth on Slants		Gram Stains		PH Final
	24 hr	72 hr	24 hrs	7 days	24 hrs	7 days		24 hr	72 hr	24 hrs	7 days	24 hrs	7 days	
B. Aerogenes	+	+	Lux	Lux	-Sh. bac	-Sh. bac	6.8	±	+	Mod	Lux	-Sh. bac	-Sh. bac	6.6
	+	+	Lux	Lux	-Sh. bac	-Sh. bac	6.8	-	+	Mod	Lux	-Sh. bac	-Sh. bac	6.2
B. Prodigiosus	-	+	Mea	Mod	-Sh. bac	-Sh. bac	6.6	-	-	Mea	-	-Sh. bac	-	6.9
	-	+	Mea	Mod	-Sh. bac	-Sh. bac	6.6	-	-	Mea	-	-Sh. bac	-	6.9
B. Dysentery Flexner	-	-	Mea	-	-Sh. bac	-	6.8	-	-	Mea	-	-Sh. bac	-	6.9
	-	-	Mea	-	-Sh. bac	-	6.8	-	-	Mea	-	-Sh. bac	-	6.9
B. Coli Communis	+	+	Lux	Lux	-Sh. bac	-Sh. bac	6.7	-	+	Mod	Lux	-Sh. bac	-Sh. bac	6.8
	+	+	Lux	Lux	-Sh. bac	-Sh. bac	6.7	-	+	Mod	Lux	-Sh. bac	-Sh. bac	6.9
B. Paratyphosus B	-	-	Mea	-	-Sh. bac	-	6.8	-	-	Mea	-	-Sh. bac	-	6.9
	-	-	Mea	-	cont.	cont.	6.9	-	-	Mea	-	-Sh. bac	-	6.9
B Paratyphosus A	-	-	Mea	-	-Sh. bac	-	6.8	-	-	Mea	-	-Sh. bac	-	6.9
	-	-	Mea	-	-Sh. bac	-	6.8	-	-	Mea	-	-Sh. bac	-	6.9
B. Aerogenes	+	+	Mod	Lux	-Sh. bac	-Sh. bac	6.8	-	±	Mea	Lux	-Sh. bac	-Sh. bac	6.6
	+	+	Mod	Lux	-Sh. bac	-Sh. bac	6.8	-	+	Mod	Lux	-Sh. bac	-Sh. bac	6.6
B. Cloacae	-	-	Mea	-	-Sh. bac	-	6.8	-	-	Mea	-	-Sh. bac	-	6.9
	-	-	Mea	-	-Sh. bac	-	6.8	-	-	Mea	-	-Sh. bac	-	6.9
B. Tuberculosis	-	-	-	Mea	-	+long bac	6.8	-	-	-	-	-	-	6.9
	-	-	-	-	-	-	6.8	-	-	-	-	-	-	6.9
B. Pyocyaneus	±	-	Lux	-	-Sh. bac	-Sh. bac	6.8	-	+	Lux	Lux	-Sh. bac	-Sh. bac	6.6
	±	-	Lux	-	-Sh. bac	-Sh. bac	6.8	-	+	Lux	Lux	-Sh. bac	-Sh. bac	6.6
B Subtilis	-	-	Mea	Mea	± bac	± bac	6.8	-	-	Mea	Mea	± bac	± bac	6.9
	-	-	Mea	Mea	± bac	± bac	6.8	-	-	Mea	Mea	I bac	± bac	6.9
Control	-	-	-	-	-	-	6.8	-	-	-	-	-	-	6.9
	-	-	-	-	-	-	6.8	-	-	-	-	-	-	6.9

Legend:
Mea = Meagre
Mod = Moderate
Lux = Luxuriant
Sh. bac = Short bacillus

DISCUSSION

The preceding tables may be summarized as follows: glycine in concentrations 0.01%, 0.05%, and 1.5% supported the growth of B aerogenes, B prodigiosus, B coli communis, B aerogenes (61), B pycocyaneus, and B subtilis. B cloacae grew in 0.05% and 1.5% glycine. B tuberculosis grew at 0.01% and 0.05% glycine but there was no apparent growth at 1.5% glycine. Growth was not visible in any concentration for B dysentery Flexner, B paratyphosus B, and B paratyphosus A.

In alanine, B aerogenes (61), B aerogenes, B prodigiosus, except at the 0.01% concentration, B coli communis, B cloacae, B tuberculosis, B pyocyaneus, and B subtilis were able to show visible growth. B dysentery Flexner and B paratyphosus B apparently failed to grow in either low or high concentration of alanine. B paratyphosus A showed no growth at 0.01% alanine and failed to sustain a meagre growth at 1.5% alanine.

Aspartic acid seemed somewhat more favorable to growth. B aerogenes, B aerogenes (61), B coli communis, B prodigiosus, B tuberculosis, B pyocyaneus, and B subtilis grew in 0.05% and 0.5% aspartic acid. Growths of the following organisms: B paratyphosus A, B paratyphosus B, and B cloacae were not maintained at 0.05% aspartic acid but all three organisms grew at 0.5% concentration. However, no growth was obtained on the agar slants for B paratyphosus A seven days after inoculation. The increased alkalinity of

the media containing aspartic acid seems to be a good indication of the utilization or breaking down of the acid. Probably the increased alkalinity was due to decarboxylization and the liberation of the amino group. The growth of B tuberculosis in this medium did not change its PH.

In the tryptophane media most of the organisms failed to sustain growth with the exception of B pyocyaneus, B tuberculosis, and B subtilis. A markedly decreased growth was shown when the cultures were incubated for seven days. B dysentery Flexner showed no growth in 0.05% and 0.75% tryptophane even after seven days incubation. B aerogenes (61) and B cloacae were reduced from a luxuriant growth, manifested on the agar slants, to a meagre growth in seven days. No growth was apparent at 0.75% tryptophane for B prodigiosus, B coli communis, B paratyphosus A, and B paratyphosus B. A meagre growth after the same period in the lower concentration was obtained with these organisms.

In tyrosine and phenylalanine the growths are similar. B aerogenes, B prodigiosus, B coli communis, B aerogenes (61), and B pyocyaneus. The failure of these results to show an inhibitory effect of tyrosine on the growth of B coli as was shown by Treece (1926) may be due to a difference in strain and in the concentration of tyrosine used. That is, the concentration of tyrosine or tyrosine split products produced, used in this experiment were not sufficient to produce inhibition. Sasaki (1914) found that B coli produced p-oxyphenylethylamine from a medium containing 0.02% tyrosine.

For both amino acids, tyrosine and phenylalanine, a

scant growth which became negative after seven days was obtained for B dysentery Flexner, B paratyphosus B, and B paratyphosus A. B subtilis grew meagerly in both acids. B cloacae gave a meagre growth at twenty-four hours in both acids but after seven days a moderate growth was obtained in tyrosine and no growth in phenyalalanine. The form of B tuberculosis used grew in 0.05% tyrosine. These results do not corroborate the theory of Long (1919) that the phenol group inhibits the growth of the tubercle bacillus. However, this may have been due to the length of time since isolation of the strain used by us and its adjustment to artificial media.

The growth of the organisms on the base medium seems variable which was probably due to the variation of ammonia nitrogen present as an impurity altho the media were aerated. However, a very minute amount of ammonia would be sufficient to maintain growth. The organisms growing well in the base medium after seven days when growth of the others was not apparent were: B aerogenes (61), B aerogenes, B coli communis, and B pyocyaneus.

SUMMARY OF PART I

1. The similarity of the growths on the amino acid media and the base medium of B dysentery Flexner, B paratyphosus A, and B paratyphosus B would suggest a failure of the organisms to utilize the amino acids used at the concentrations employed in the experiment.

2. B aerogenes (61), B aerogenes, B coli communis, B pycocyaneus, and B subtilis were the most vigorously growing organisms in all of the media used.

3. The reduction in tryptophane media of the moderate and luxuriant growths at twenty-four hours of B aerogenes (61), B aerogenes, B prodigiosus, B dysentery Flexner, B coli communis, B paratyphosus A, B paratyphosus B, and B cloacae was noticeable seven days after inoculation. This may have been due to the toxic products produced in the decomposition of tryptophane by these organisms or possibly to the lack of utilization of tryptophane. It seems hardly probable that the tryptophane was toxic as a whole because luxuriant growths were obtained with these organisms at twenty-four hours.

4. No toxic or inhibitory action of alanine, glycine, aspartic acid, tyrosine, and phenylalanine was apparent with the concentrations of amino acids for the organisms used under the conditions of the experiment.

5. Of the amino acids used, aspartic acid seemed the most readily utilized by the organisms used.

PART II

In order to determine more definitely the effect of the amino acids upon the bacteria and since the growth rate method has not been previously applied to this problem, the rate of growth of certain bacteria in certain amino acid media was quantitatively determined.

While studying the growth of bacteria, Muller (1895) was the first to have recognized the latent period in the growth of bacteria. Heheworth (1901) maintained that the lag phase was shorter for B coli than for B typhosus. He also ascertained that the duration of lag was less in culture medium to which the organism was adapted. The lag phase was studied by Rahn (1906) who found the lag shortened with a large inoculum. Penfold (1914) corroborated Rahm and noted, too, that the length of the lag varied in different media. Barber (1908) was the first to show that the lag phase could be eliminated by inoculating an actively dividing coli bacillus into medium to which it is accustomed. Chesney (1916) divided the growth of bacteria into four phases:

1. Latent period including the time of seeding to the time at which maximum rate of growth occurs.
2. Logarithmic period in which multiplication follows the law of geometric proportions.
3. Stationary period in which there is no increase in the number of viable organisms.
4. Period of decline in which the number of organisms decreases.

Later Buchanan (1918) further divided the growth of bacteria into seven periods:

1. The initial stationary stage.
2. Lag or positive growth acceleration.
3. Logarithmic growth.
4. Negative growth acceleration.
5. Maximum stationary phase.
6. Accelerated death phase.
7. Logarithmic death phase.

Salter (1919) showed that the greatest effect of brilliant crystal violet and brilliant green in inhibiting concentrations was in the lengthening of the lag period. Tanner and Wallace (1924) found by using thermophilic bacteria that a lowered temperature increased the lag period, decreased the growth, and delayed the period of negative acceleration. Treece (1926) in comparing the growth curves in cystine and phenylalanine of B aerogenes notes that in phenylalanine a much steeper slope and greater growth is produced than in cystine.

The following experiment, then, is to determine quantitatively what effect certain amino acids have upon the growth of certain bacteria.

METHOD

The quantitative estimation by growth rates of the growth of the organisms in the amino acid media was made as follows:

The organisms used were B aerogenes, B coli communis, B paratyphosus B, and B dysentery Flexner. The amino acids used were glycine, aspartic acid, and tryptophane.

The organisms were standardized by five successive twenty-four hour inoculations on plain agar slants. Each time a standard loop was touched to the growth and one stroke made over the surface of the new slant. For inoculation into the liquid media a nine to twelve hour slant was used. Before inoculation in the media, the organisms were emulsified in 10cc sterile distilled water from which ammonia had been removed by standing over permutit at least twelve hours. This suspension was then further diluted, using the de-ammonized sterile distilled water in 99cc and 9cc water blanks, to a final dilution of 1-1million. This dilution was generally found by plating 1cc in agar plates to contain 500 organisms per cubic centimeter. One cc of the final dilution, approximately 500 organisms, was inoculated into each of the flasks.

The base medium used in Part I was used here. For the growth rate of each organism in each amino acid the following media at least were used:

1. Base medium and 1% Difco bactopeptone.
2. Base medium plus 1% Difco bactopeptone, plus amino acid.

3. Base medium plus amino acid.

4. Base medium.

If more than one concentration of amino acid was desired the peptone-amino acid-base medium was also run.

The method of deammonization and sterilization was the same as in Part I. The media containing peptone, however, were not deammonized.

Inverted 50cc of 100cc beakers were placed over the flasks containing the peptone media and sterilized with the media. Cotton in the bottom of the beaker, as used by Tanner and Wallace (1924), was not found necessary. For the media containing an amino acid as the sole source of nitrogen and for the base medium, sterile beakers wer e placed over the top of the flask after deammonization.

Gram stains on the organisms were made before the slants were emulsified for dilution and inoculation. Agar slants were inoculated after the organisms had grown 6, 12, 24, 48, and 72 hours in the culture media. Gram stains were made from the slants to test for contamination.

The growth of the organisms was estimated by plating 1cc of the medium or 1cc of diluted medium in plain agar. Two plates were made for each dilution. Sterile 99cc water blanks were made for the dilutions and were cleaned, refilled and sterilized before they were used again so that there would be no chance of carrying over toxic substances. The plates were incubated at 37°C for 48-72 hours and then counted using the Jeffer's Plate counter.

RESULTS

In the following tables the plate counts are recorded as the logarithms of the number of organisms per cc of the culture medium.

Table VIII. Growth Rates of B. Aerogenes in Aspartic Acid

Hours Incubation	1% Peptone Base	0.5% Aspartic Acid — 1% Peptone Base	0.05% Aspartic Acid 1% Peptone Base	0.5% Aspartic Acid Base	0.05% Aspartic Acid Base	Base
0	2.2304	2.0969	2.1761	2.0414	2.0792	2.0969
1	2.2041	2.0792	2.1761	2.1139	2.1461	2.1461
2	2.3010	2.1461	2.3522	2.0792	2.2175	2.1139
3	3.2304	3.3010	3.3222	2.2041	2.0969	2.0000
4	4.2553	4.2553	4.3010	2.5682	2.2430	2.3010
5	5.2041	5.1761	5.3010	2.8751	2.5441	2.3979
6	5.8751	5.9294	6.0000	3.1139	3.0000	---
7	6.8129	6.7782	6.8451	3.7404	3.4771	2.8751
8	7.4771	7.5185	7.4771	4.0969	3.8751	3.1761
9	8.3010	8.3010	8.3010	4.3979	4.3522	3.6990
10	8.6990	8.6021	8.6021	4.8451	4.6021	3.9294
11	8.7404	8.7782	8.7782	5.1761	4.8751	4.2041
12	8.7782	8.7782	8.7782	5.6021	5.2553	4.5441
24	8.8751	8.9031	8.9542	8.6021	8.4472	6.1761

Table IX. Growth of B. Aerogenes in Tryptophane Media

Hours Incubation	1% Peptone Base	0.75% Tryptophone 1% Peptone Base	0.75% Tryptophone Base	Base
0	2.2304	2.0000	2.0212	2.0969
1	2.2041	1.9542	2.0414	2.1461
2	2.3010	2.0792	2.0000	2.1139
3	3.2304	2.5441	2.0414	2.0000
4	4.2553	3.4150	1.6532	2.3010
5	5.2041	4.2041	1.6990	2.3979
6	5.8751	4.9542	1.4771	
7	6.8129	5.6532	1.3979	2.8751
8	7.4771	6.3979	1.0000	3.1761
9	8.3010	7.1761	1.0000	3.6990
10	8.6990	8.1761		3.9294
11	8.7404	8.3979	.6990	4.2041
12	8.7782	8.5441	.3010	4.5441
24	8.8751	8.6532	8.1761	6.1761

Table X. Growth of B. Aerogenes in Glycine Media

Hours Incubation	1.5% Glycine 1% Peptone Base	1% Peptone Base	1.5% Glycine Base	Base
1	1.0000	.8451	1.0792	.7782
2	1.6532	1.3424	1.1139	.7782
3	2.2553	2.4314	1.2788	.6990
4	3.3010	3.1761	2.0000	.7782
5	4.4771	4.5185	2.4771	1.1761
6	5.1761	6.2553	3.0000	1.1761
7	6.3010	6.6021	3.3010	1.3010
8	7.3979	7.2041	3.7782	1.1761
9	7.5441	8.1761	4.4771	2.3979
10	7.6021	8.6021	5.0000	2.6990
11	7.9031	8.4771	5.6990	2.7782
12	8.4771	8.4771	6.7782	3.0000
24	8.3979	8.6532	7.1761	6.8129
36	8.4771	8.5441	7.0792	7.1761
48		8.1461		6.7782
72	8.3802		7.0000	6.6021
96	8.1761	7.8129	6.8751	6.6990
120	7.5441	7.6021	6.3979	6.1761
144	7.3979	7.5441	6.2430	6.1761

Table XI. Growth Rates of B. Coli Communis in Aspartic Acid Media

Hours Incubation	1% Peptone Base	0.5% Aspartic Acid 1% Peptone Base	0.05% Aspartic Acid 1% Peptone Base	0.5% Aspartic Acid Base	0.05% Aspartic Acid Base	Base
0	2.1139	2.0792	2.0414	2.1461	2.0792	2.0607
1	2.0792	2.1761	2.1139	2.9069	2.0000	2.0000
2	2.3979	2.5441	2.4771	1.9031	2.0000	1.9031
3	3.3979	3.4771	3.5051	2.0969	2.0000	1.9294
4	4.0792	4.1761	4.3617	2.6532	2.0969	2.0000
5	4.7482	4.8129	5.0000	2.7782	2.1761	1.9031
6	---	5.6990	6.0414	3.6021	2.2430	1.8751
7	6.0000	6.5441	6.7404	4.3010	2.4771	1.9542
8	7.3617	7.2553	7.5441	4.7782	2.7404	2.0414
9	8.2304	8.0969	7.3979	5.3802	3.2304	2.1139
10	8.6532	8.6532	8.7404	5.7404	3.4771	2.1761
11	8.6021	8.8451	8.6990	6.3979	4.1139	3.7782
12	8.8129	8.9031	8.8751	7.0000	4.3424	4.3979
36	9.0000	---	---	8.3979	8.0000	6.6021
48	9.0000	9.0792	9.0969	8.5441	8.1761	6.6532

Table XII. Growth Rates of B. Coli Communis in Tryptophane Media

Hours Incubation	1% Peptone Base	0.75% Tryptophane 1% Peptone Base		0.05% Tryptophane 1% Peptone Base	0.75% Tryptophane Base	0.05% Tryptophane Base	Base
1	2.0969	2.1761	2.1761	2.1761	2.2430	2.2304	2.1761
2	2.4771	2.3522	2.3979	2.6021	2.2430	2.2430	2.1761
3	3.3979	3.0969	3.1461	3.3979	2.1761	2.2041	2.0969
4	4.3010	3.8451	3.9031	4.3010	2.1139	2.3522	2.1139
5	4.9777	4.5441	4.6532	5.0607	2.2041	2.6532	2.1139
6	6.1903	5.5798	5.6532	6.2553	2.3010	3.2430	2.1139
7	6.9031	6.3522	6.3802	7.0792	2.4771	3.6990	2.1761
8	7.6532	6.9031	7.0000	7.6990	2.6990	4.0000	2.3010
9	8.2430	7.1761	7.4771	8.3010	3.0792	4.3522	2.3010
10	8.8782	8.0969	8.2553	8.7782	3.3010	4.6284	2.4771
11	8.9777	8.6532	8.6990	8.9542	3.4771	5.0000	2.5441
12	8.9777	8.7782	8.7782	8.9777	3.7782	5.4771	2.6990
24	---	---	---	---	7.8451	7.2304	4.6990

Table XIII. Growth Rates of B. Coli Communis in Glycine Media

Hours Incubation	1% Peptone Base			1.5% Glycine 1% Peptone Base			0.05% Glycine 1% Peptone Base	1.5% Glycine Base		0.05% Glycine Base	Base
0	1.1461	2.1139	2.0792	2.1461	1.8688	1.8041	1.8435	1.5441	1.0792	1.6232	1.4771
1	1.1139	2.0792	1.8451	2.0792	1.6532	1.2304	1.7924	1.5798	1.0000	1.6435	1.6232
2	1.6232	2.3979	2.3010	2.3979	2.0969	1.3222	2.3010	1.5315	1.1761	1.6021	1.5315
3	2.5798	3.3979	3.0000	2.7404	2.7404	2.0000	3.0969	1.4771	1.1761	1.6021	1.8195
4	3.5051	4.0792	4.1761	2.6990	2.9542	2.1461	4.0969	1.7076	1.3979	2.2430	1.5441
5	4.4771	4.7482	5.0000	2.3010	---	2.3010	5.1303	2.1761	1.6021	2.6532	1.6021
6	5.4914	---	6.1614	2.2553	---	less than 4,000	6.2788	2.6532	2.0000	3.1303	1.6021
7	6.5441	6.0000	7.2553	2.1761	---	"	7.1761	3.0000	2.3010	3.6990	1.6990
8	7.5185	7.3617	8.0969	2.0969	---	"	7.9031	3.6021	2.6532	4.2430	1.7782
9	8.2041	8.2304	8.6021	1.8751	---	"	8.5740	3.8751	3.2304	4.5119	2.0000
10	8.6532	8.6532	8.6232	1.4771	---	"	8.4771	4.1761	3.6532	5.1614	2.3522
11	9.0702	8.6021	8.8451	1.4771	---	"	8.7782	4.6767	4.0000	5.4472	2.5185
12	9.1761	36 Hours 8.8129	8.7782	1.3010	---	"	8.7404	5.0607	4.1761	5.6990	2.6990
24	9.3979	9.0000	8.8451	3.3010	---	3.1761	8.8751	6.8990	--	7.3010	6.6021
48	9.1761	9.0000	9.0969	5.6990	7.2041*	3.1461	9.3979	6.9031	7.4314	7.[illegible]021	6.4314
72	9.1761		9.0000	7.4314	7.3010*	7.6532	8.7782	6.6021	7.3010	8.0000	6.0000

* Two flasks erroneously inoculated, the one used became contaminated, so flask II was substituted at 48 hours. It was clear at 12 hours.

Table XIV. Growth Rates of B. dysentery Flexner in Aspartic Acid Media

Hours Incubation	1% Peptone Base	0.5% Aspartic Acid 1% Peptone Base	0.05% Aspartic Acid — 1% Peptone Base	0.5% Aspartic Acid — Base	0.05% Aspartic Acid — Base	Base
0	1.8451	1.8451	1.9031	1.6021	1.8129	1.5441
1	1.9031	1.9294	1.9031	1.5441	1.6532	1.3979
2	1.8129	2.0000	1.8451	1.2041	1.2553	.9031
3	1.8451	2.1761	2.2041	1.1761	1.1761	.3010
4	2.0000	2.3424	2.0414	1.0792	1.0792	.6021
5	2.3979	2.4772	2.1761	1.5441	1.3010	.6021
6	2.6021	2.8451	2.5441	1.4771	1.3424	.6990
7	2.9031	3.0414	2.6990	1.5185	1.3010	.6990
8	---	3.3617	2.9031	1.4472	1.3617	.4771
9	3.1761	3.5441	3.0000	1.6990	1.3010	.3010
10	3.4771	3.6990	3.2304	1.6532	1.1761	0
11	---	3.9542	3.5441	1.7404	1.1761	.0000
12	3.7782	4.1761	3.6990	1.7782	1.3010	.0000
24	8.4472	8.8451	8.5740	2.2430	.8451	0

Table XV. Growth Rates of B. dysentery Flexner in Tryptophane

Hours Incubation	1% Peptone Base	0.75% Tryptophane 1% Peptone Base		0.05% Trypto Phane 1% Peptone Base	.75% Trypto-Phane Base	0.05% Trypto-phane Base	Base
1	2.7782	2.8129	2.8129	2.8129	2.6990	2.7959	2.7782
2	2.8129	2.8451	2.8451	2.8451	2.6990	2.7404	2.7782
3	3.0792	3.3522	3.3802	3.3979	2.7404	2.7404	2.7959
4	3.6021	3.9031	3.8751	3.6532	2.6990	2.6990	2.6990
5	4.0969	4.6021	4.5441	4.1761	2.6284	2.6990	2.6532
6	4.5010	5.1614	5.1139	4.6532	2.6990	2.7404	2.6990
7	4.8129	5.6232	5.5563	5.1461	2.6532	2.7404	2.6532
8	5.3010	6.3016	6.1761	5.6532	2.6532	2.7202	2.6021
9	5.7782	7.0000	6.8451	6.0414	2.6990	---	2.4771
10	6.2430	---	---	---	---	---	---
11	6.6990	7.9031	7.8751	7.1461	2.7782	2.6990	2.1761
12	7.0000	8.4771	8.3979	8.0969	2.7762	2.4771	2.0000
36	8.0414	8.6990	8.6532	8.8751	---	0	0
60	---	---	---	---	2.1761	0	0

Table XVI. Growth Rates of B. dysentery Flexner in Glycine

Hours Incubation	1% Peptone Base	1.5% Glycine 1% Peptone Base		0.05% Glycine 1% Peptone Base	1.5% Glycine Base	0.05% Glycine Base	Base
0	.8451	.9031	1.9294	.9031	.7782	.6990	.6990
1	1.1761	.9542	1.9542	.6990	1.0000	.6990	.6990
2	1.2041	.7782	2.0000	.8451	.6990	.4771	.6990
3	1.4150	1.3979	1.9542	1.3010	.3010	.6021	.4771
4	1.6021	2.1761	2.3979	1.6990	.3010	.6990	.4771
5	2.2304	2.5185	2.5119	2.0792	.4771	.6021	.4771
6	2.5441	3.0969	3.0000	2.5441	.0000	.4771	.0000
7	2.8129	3.6990	3.3979	2.9031	.0000	.3010	.3010
8	---	4.0969	3.5441	3.6532	.3010	.4771	.0000
9	3.7782	4.6990	3.9243	4.3010	.0000	.3010	.3010
10	4.3979	5.4314	4.3979	4.4771	.3010	.0000	.3010
11	4.9031	5.7782	4.6435	5.3010	0	0	.0000
12	---	6.2430	4.7404	5.6021	0	0	.0000
24	---	---	8.1761	---	0	0	0
48	---	---		8.3010	0	0	2.6021
72	---	---		---	0	0	5.3010

Table XVII. Growth Rates of B. Paratyphosus B.

in Aspartic Acid Media

Hours Incubation	1% Peptone Base	.5% Aspartic Acid — 1% Peptone Base	0.05% Aspartic Acid 1% Peptone Base	0.5% Aspartic Acid Base	0.05% Aspartic Acid Base	Base
0	1.7404	1.6990	1.8129	1.7404	1.7782	1.8451
1	1.7782	1.7782	1.8129	1.7782	1.7782	1.8129
2	1.9031	1.7404	1.8751	1.8129	1.7782	1.6990
3	2.2041	2.2553	2.2553	2.0000	1.7782	1.6990
4	3.9777	3.0000	3.0414	2.3010	1.8451	1.4771
5	3.6990	3.7782	3.7782	2.3979	1.7782	1.5441
6	4.3522	4.6021	4.4771	2.7782	1.8129	1.4771
7	5.2553	5.3771	5.3979	3.3617	1.7404	1.5441
8	5.8129	6.0000	6.0969	---	---	---
9	6.5441	6.6021	6.6532	3.6532	1.5441	1.4771
10	7.1461	7.1761	7.2041	3.9777	1.6021	1.3979
11	7.7404	7.8451	7.9031	4.4771	1.6532	1.3979
12	8.3522	8.3979	8.4771	5.3979	1.6021	1.4771
24	8.7404	8.8129	8.7959	5.7404	1.6021	2.3010
72	8.7782	8.6532	8.8129	6.1761	3.3979	4.4314

Table XVIII. Growth Rates of B. Paratyphosus B.

in Tryptophane Media

Hours Incubation	1% Peptone Base		.5% Tryptophane 1% Peptone Base	.05% Tryptophane 1% Peptone Base		.5% Tryptophane Base	.05% Tryptophane Base	Base
1	2.0969	2.2430	2.0000	2.3010	2.3522	2.0000	2.2553	2.0969
2	2.1761	2.0000	2.0000	2.3522	2.3979	1.8751	2.0000	1.9031
3	2.5441	3.0792	2.4771	3.1139	---	1.9542	1.9542	2.0000
4	3.0792	4.0000	3.0000	3.7782	3.9031	2.0969	2.0969	2.0969
5	3.9542	4.5031	4.0000	4.6021	4.4771	2.0792	2.0969	2.3010
6	5.0000	5.6021	5.0792	5.6021	5.5682	2.0000	2.0000	2.3979
7	5.4771	6.3617	5.6021	6.3979	6.3979	2.0000	2.0000	2.8451
8	6.0969	6.9542	6.1139	---	6.9542	2.1139	2.0000	3.0792
9	6.6021	7.5441	6.6021	----	7.3974	2.0000	2.2041	3.5441
10	7.3010	8.0000	7.1461	---	7.9031	2.2041	2.3010	3.7782
11	8.0792	8.6990	8.0000	---	8.6532	2.1761	---	---
12	8.4771	8.9031	8.3979	---	8.8751	2.0000	2.4771	4.2553
36	8.7404	---	8.6990	---	---	2.0000	More than 4,0000	6.3802

Table XIX. Growth Rates of B. Paratyphosus B.

in Glycine Media

Hours Incubation	1% Peptone Base	1.5% glycine 1% Peptone Base	0.05% glycine 1% Peptone Base	1.5% glycine Base	0.05% glycine Base	Base
0	More than 2,000	More than 2,000	2.5441	More than 2,000	2.4771	More than 2,000
1	"	"	2.5682	"	2.3979	"
2	"	"	2.7559	"	2.3979	"
3	More than 4,000	3.1761	3.5441	"	2.3010	"
4	"	2.7404	4.6532	"	2.3010	"
5	"	2.3010	---	"	2.3010	"
6	"	2.0000	6.0000	"	2.2304	"
7	"	1.8451	6.6812	"	2.2041	"
8	"	1.7782	7.6232	"	2.3010	"
9	"	1.7782	8.1761	"	---	"
10	"	2.0000	8.4771	"	---	"
11	"	2.0792	8.7404	"	---	"
12	"	2.2553	8.7482	"	---	"
24	"	7.8451	9.0792	4.3802	---	2.6990
48	0.5441	7.8771	---	5.1761	5.1461	---

Table XX. Growth of B. Paratyphosus B. in Glycine Media

Hours Incubation	1% Peptone Base	1.5% Glycine 1% Peptone Base	0.05 Glycine 1% Peptone Base	1.5% Glycine Base	.05% Glycine Base	Base
0	2.5563	2.5441	2.5441	2.4771	2.5441	2.5798
1	2.6021	2.5682	2.6021	2.4771	2.5563	2.5682
2	2.6990	2.5911	2.6335	2.4771	2.5441	2.5798
3	3.3424	2.2304	3.2553	2.4472	2.5682	2.5441
4	3.6021	.8451	3.5563	2.3979	2.5441	2.5682
5	4.7924	.4771	4.7482	2.3979	2.4771	2.5441
6	5.0969	.3010	5.2552	2.3617	2.4771	2.4771
7	5.7782	less than 2,000	5.8573	2.3010	2.5315	2.4771
8	6.3424	less than 2,000	6.4771	2.3424	2.5441	2.2553
9	7.0792	less than 2,000	7.2553	2.3010	2.6990	2.4771
10	7.5441	less than 2,000	7.6021	2.2041	2.6990	2.4771
11	8.3010	less than 2,000	8.4771	2.2553	2.7404	2.5441
12	8.6990	less than 2,000	8.7782	2.1761	2.7782	2.4771
24	8.7782	con-taminated	8.7404	2.1761	3.0000	2.4472
48	8.6990		8.7782	less than 2,000	5.2553	2.1139

PLATE I. GROWTH CURVES OF B. AEROGENES IN GLYCINE, TRYPTOPHANE AND ASPARTIC ACID.

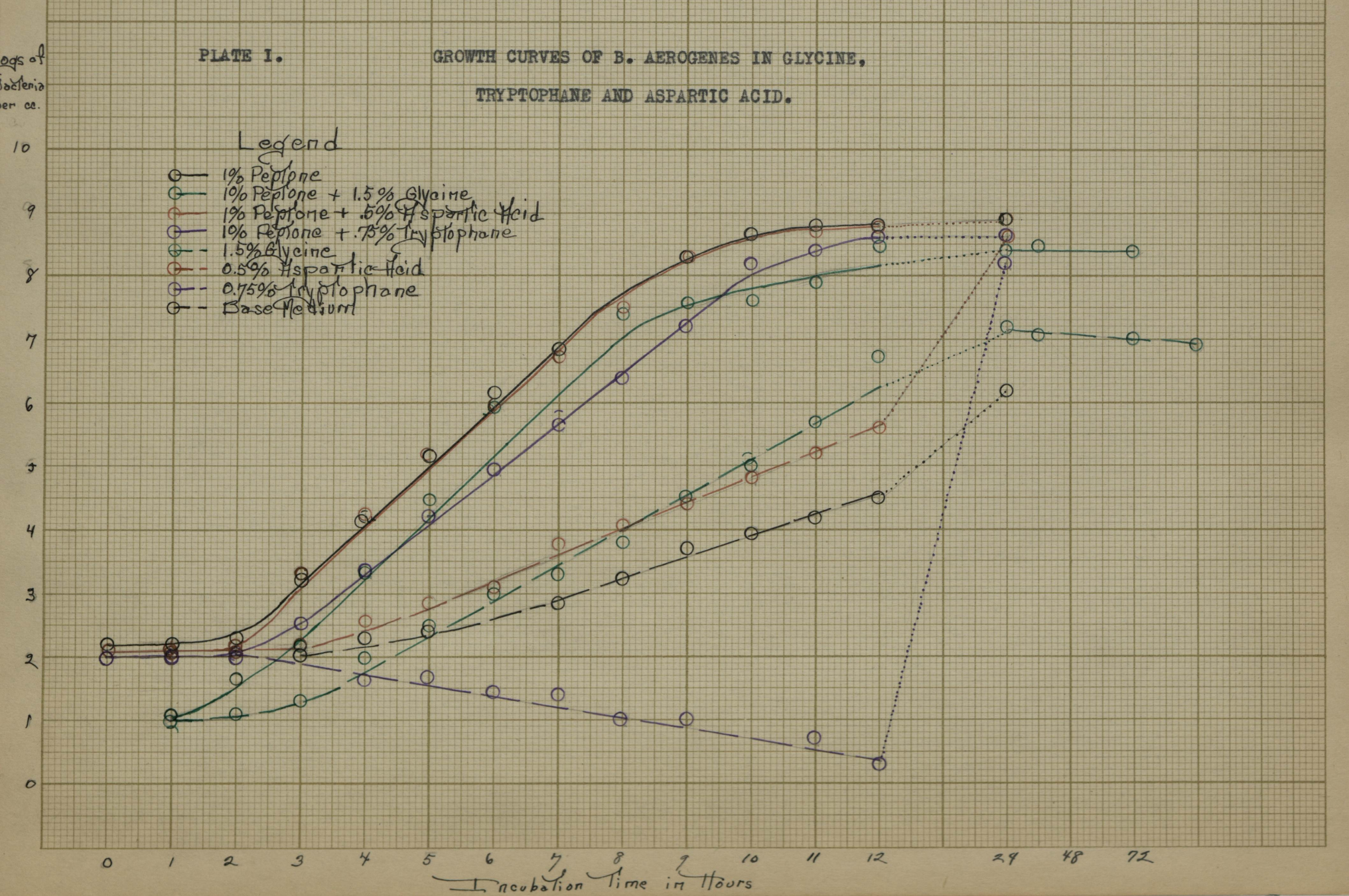


PLATE II. GROWTH CURVES OF B. COLI IN GLYCINE, TRYPTOPHANE, AND ASPARTIC ACID.

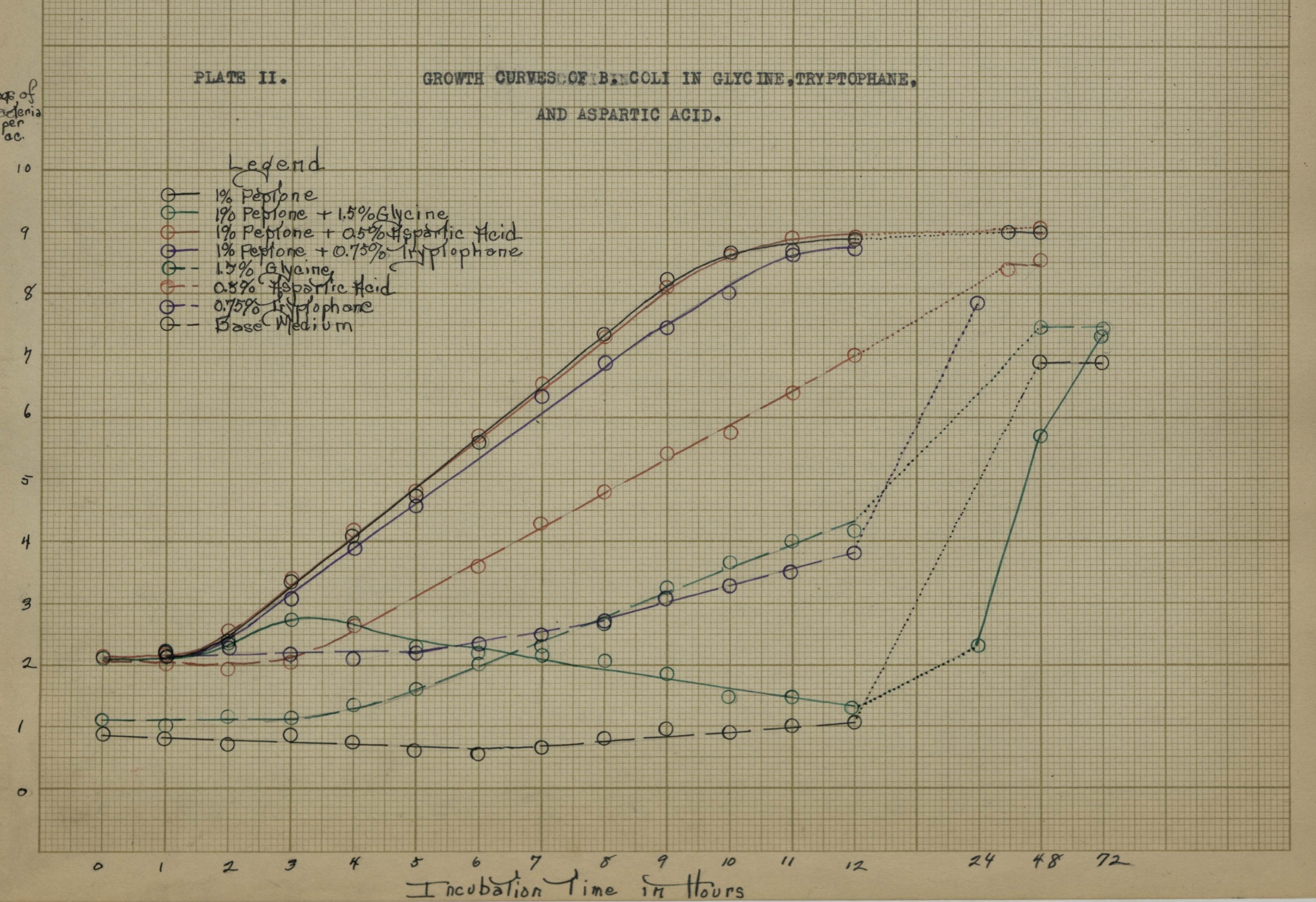


PLATE III. GROWTH CURVES OF B. DYSENTERY FLEXNER IN GLYCINE, TRYPTOPHANE, AND ASPARTIC ACID.

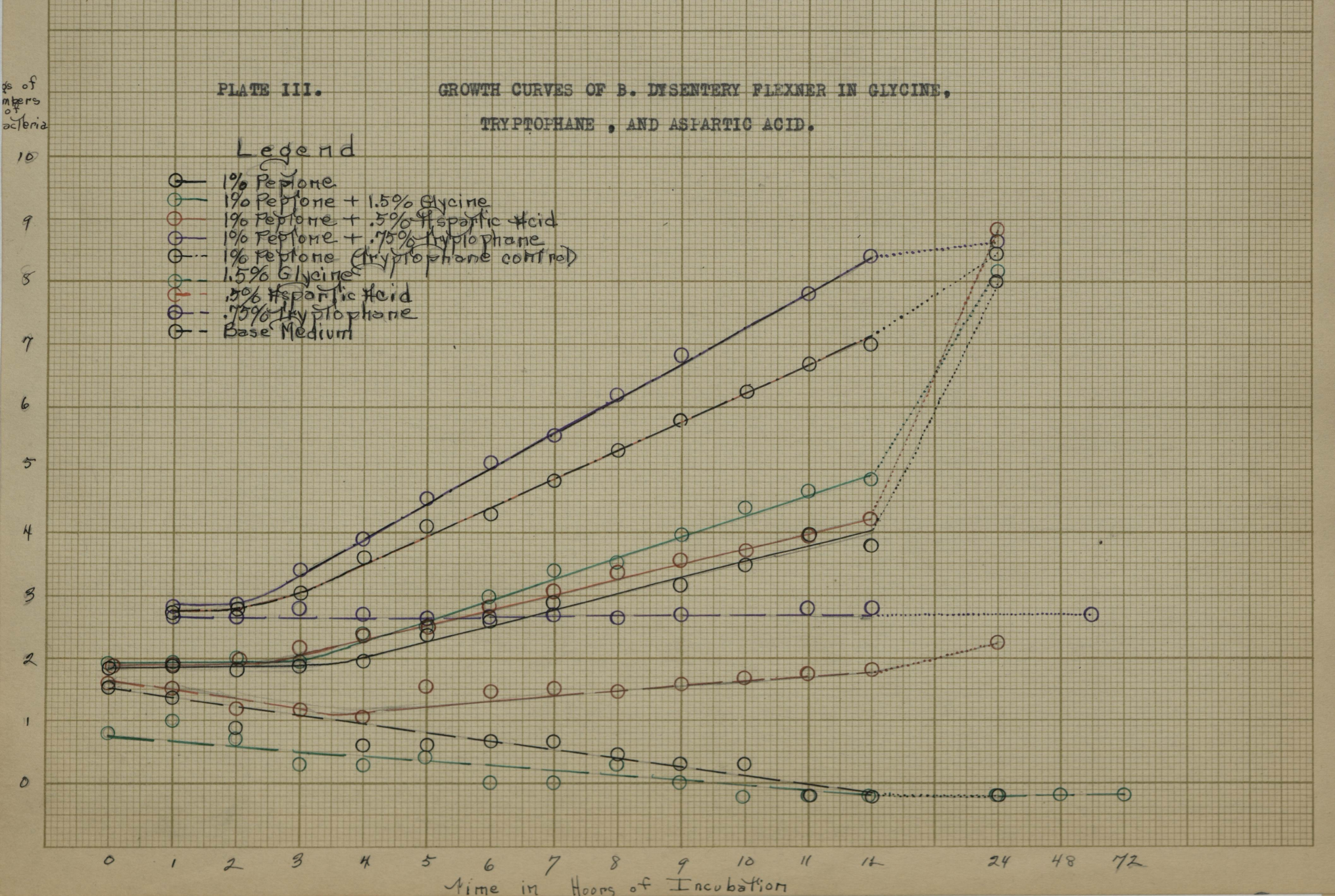


PLATE IV. GROWTH CURVES OF B. PARATYPHOSUS B IN GLYCINE, TRYPTOPHANE, AND ASPARTIC ACID.

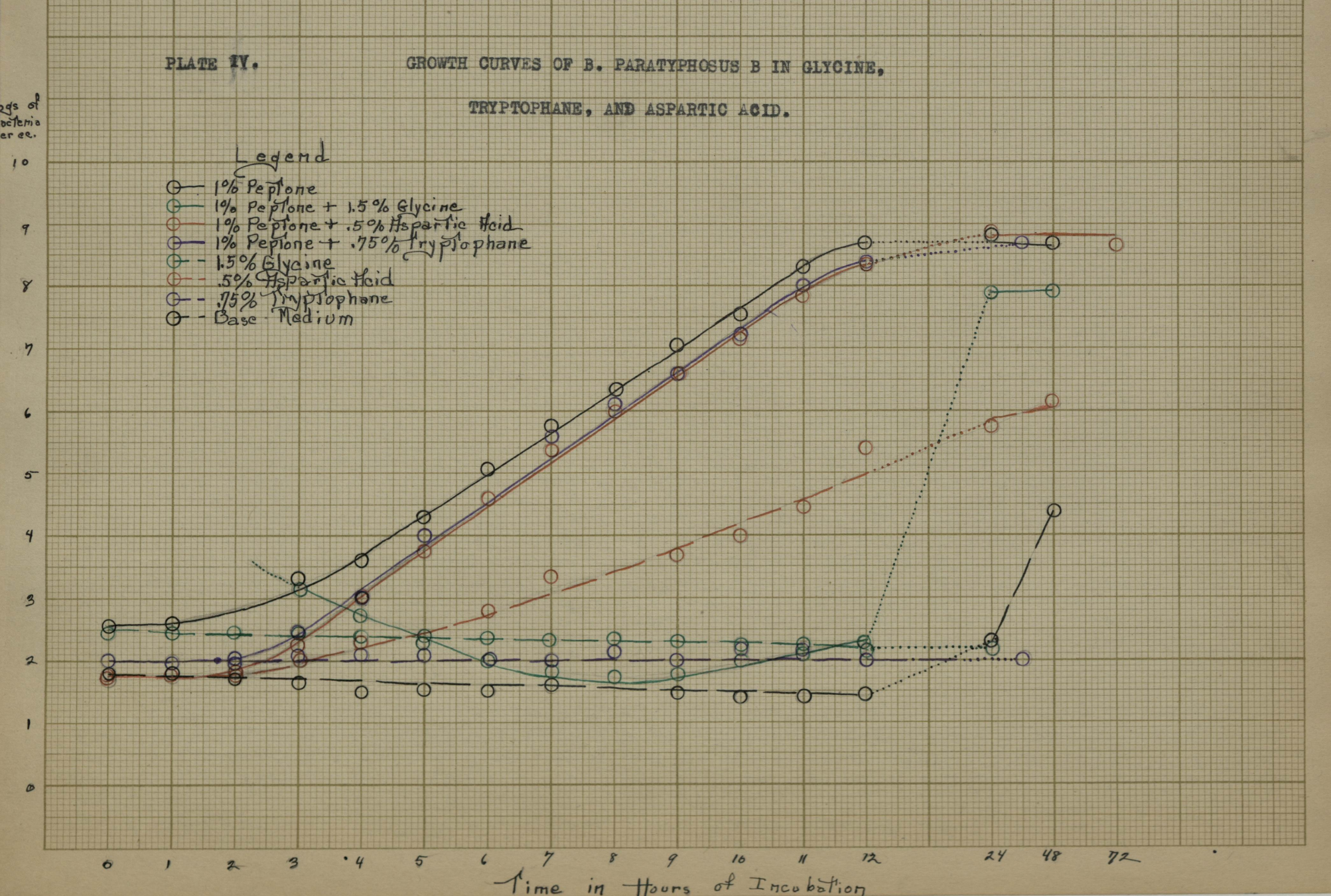


PLATE V. GROWTH CURVES OF B. PARATYPHOSUS B, B. AEROGENES, B. DYSENTERY FLEXNER, AND B. COLI IN GLYCINE.

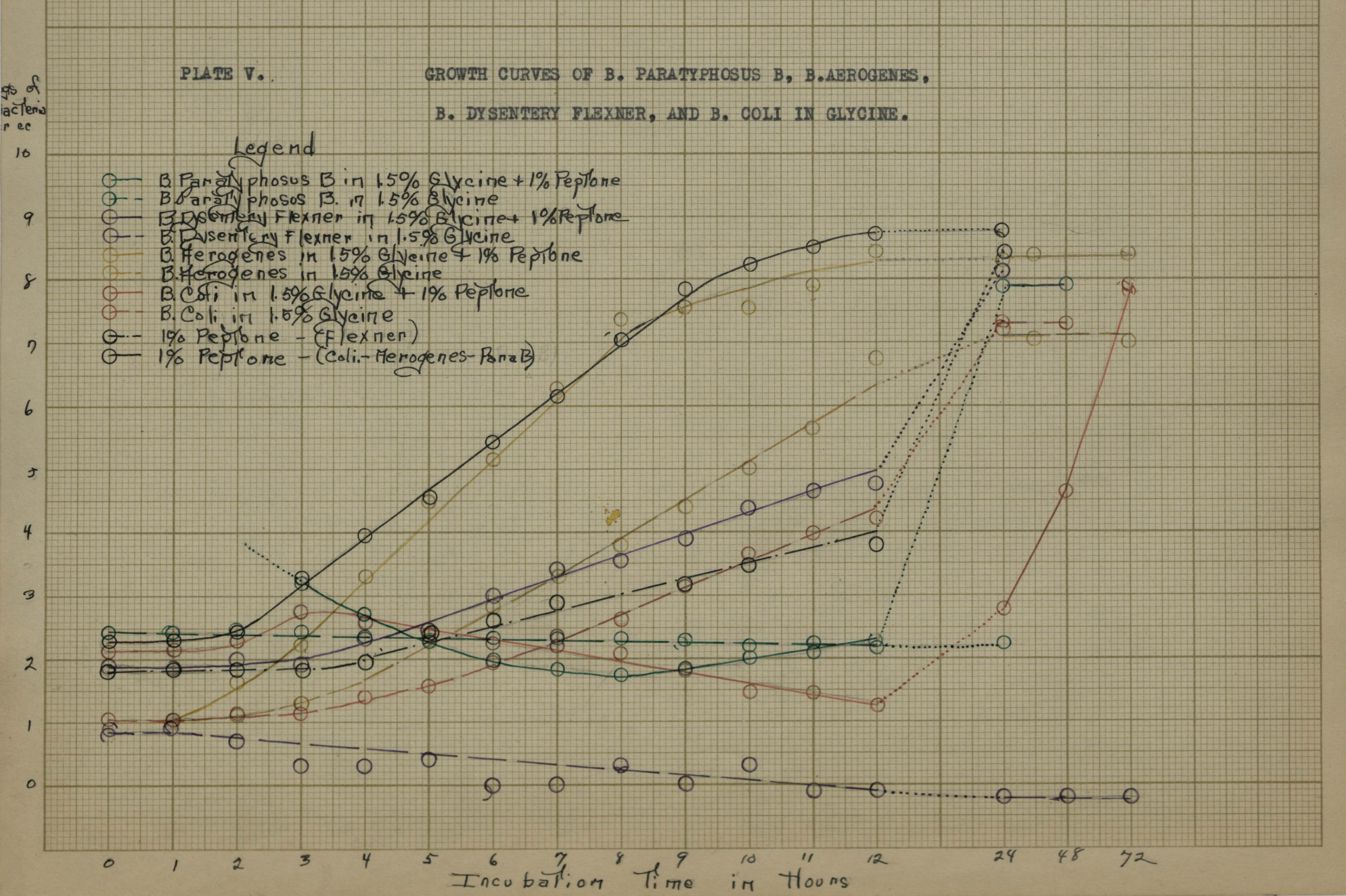


PLATE VI. GROWTH CURVES OF B. PARATYPHOSUS B, B. AEROGENES,

B. DYSENTERY FLEXNER, AND B. COLI IN ASPARTIC ACID.

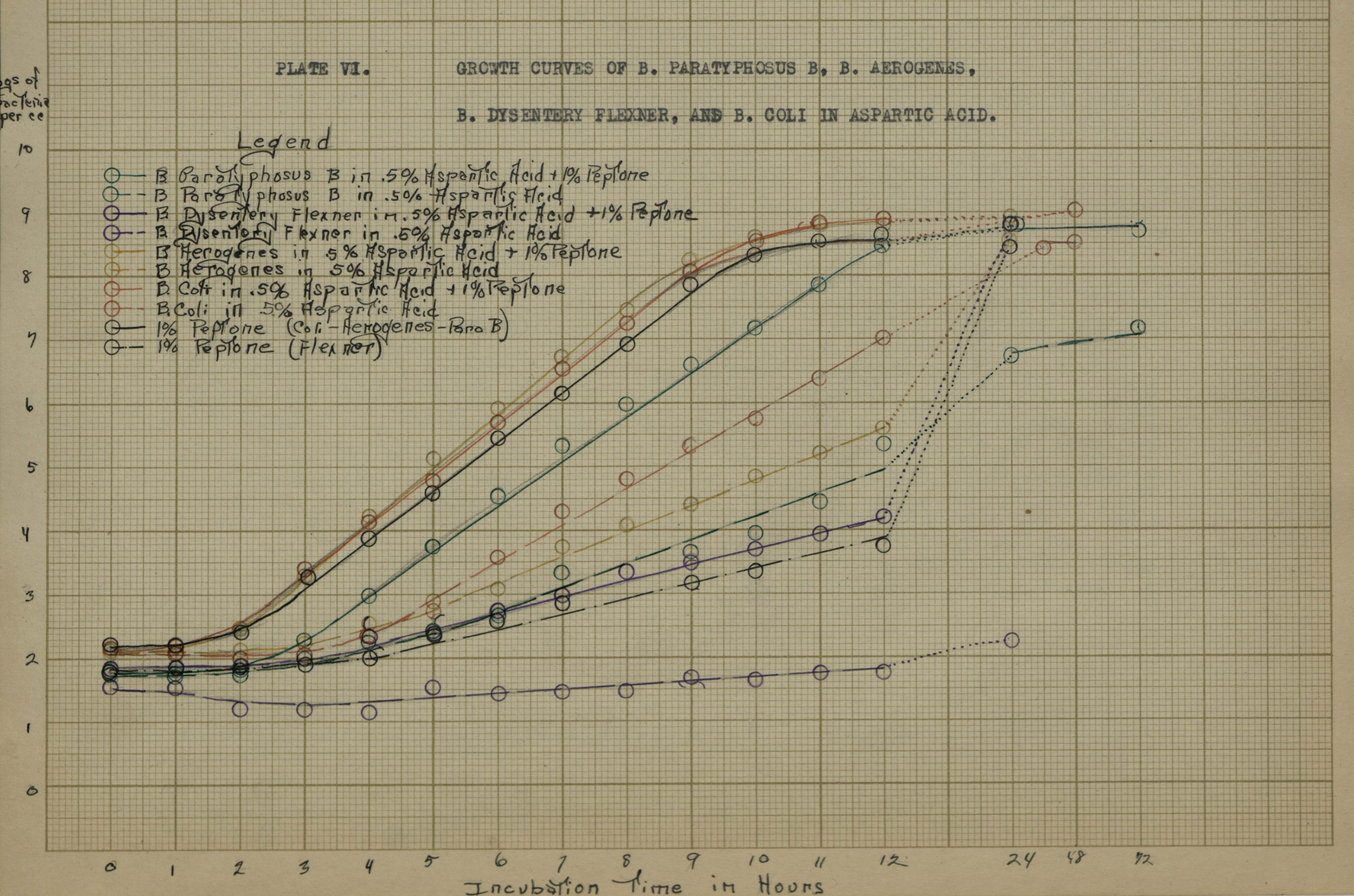
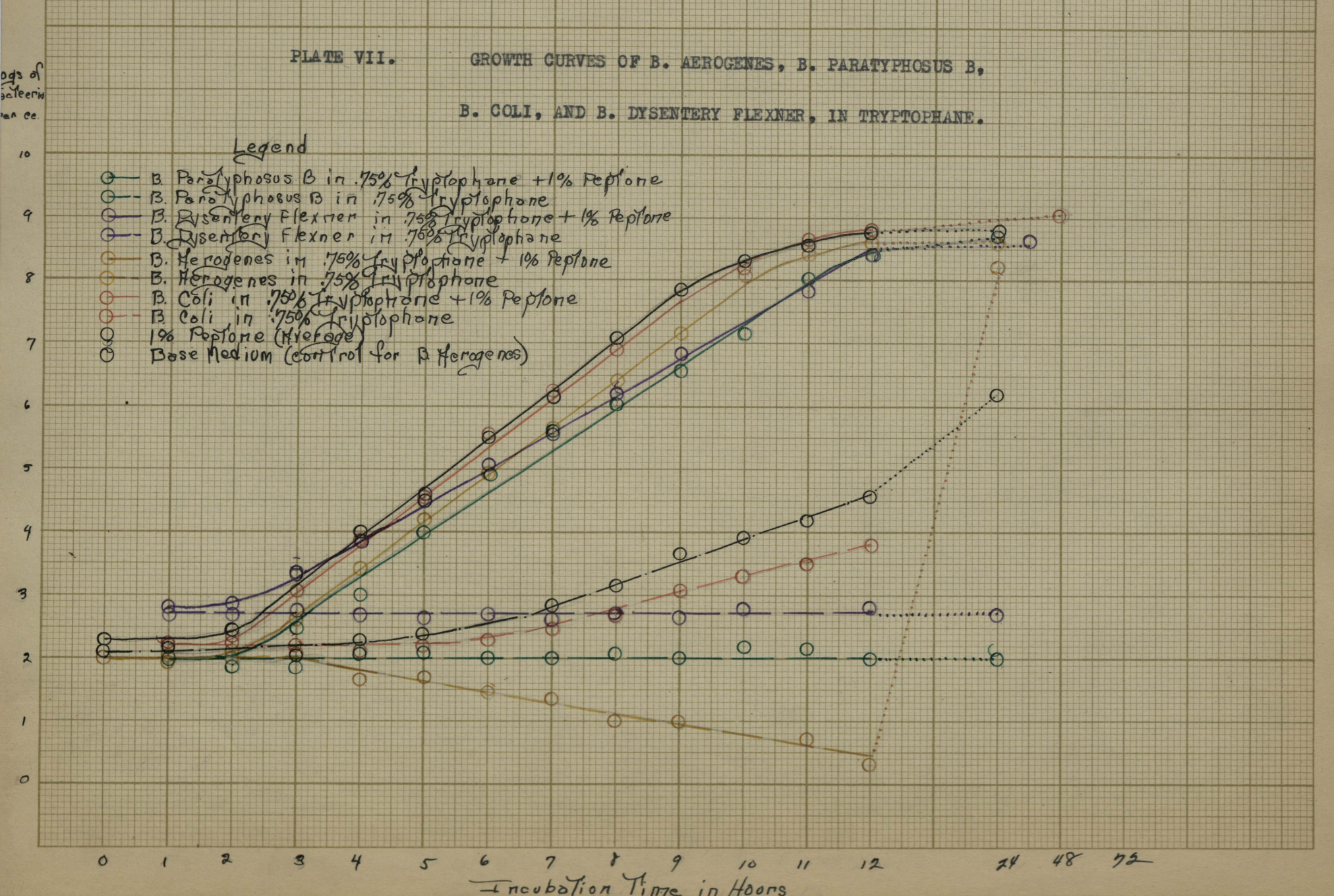


PLATE VII. GROWTH CURVES OF B. AEROGENES, B. PARATYPHOSUS B, B. COLI, AND B. DYSENTERY FLEXNER, IN TRYPTOPHANE.



DISCUSSION

Many suppositions have arisen as to the probable effect of amino acids on bacteria. One theory is that amino acids are toxic of themselves or that the metabolic products are toxic for the bacteria. Wyon and McLeod (1923) portend that some amino acids produce an inhibitory effect ranging from complete inhibition to partial or no inhibition. Treece (1926) suggests the selective inhibition of bacterial growth by amino acids. He showed that tyrosine in 0.1% concentration inhibited the growth of B coli and delayed the production of H_2S from cystine. This effect was not apparent with B aerogenes and B paratyphosus B. The possible explanation given is that B coli splits phenol from the tyrosine molecule while B paratyphosus B and B aerogenes do not.

Then, again, the organisms may be unable to utilize the nitrogen when bound in the amino acid molecule. Furthermore, some amino acids might produce a stimulating effect. This is closely allied with the theory of selective action of bacteria. Treece (1926) has demonstrated that bacteria have shown a selective preference for certain amino acids. By comparison of the growth rates of bacteria in a synthetic medium, he has found that the curve of B aerogenes in phenylalanine and in glycine is steeper and higher than in cystine. Further, he found the production of H_2S, an index of the utilization of cystine, delayed when glycine or phenylalanine was added to the synthetic medium containing cystine.

The data recorded above should elucidate to some extent the question of the effect of the amino acids used on the bacteria employed under the conditions of the experiment. The accompanying graphs have been included to facilitate the interpretation of the data.

In examining the tables and the graph, Plate I, of the effect of 1.5% glycine, 0.5% and 0.05% aspartic acid, and 0.75% tryptophane in peptone and peptone-free media on B aerogenes, we find that in the peptone - amino acid - base medium the growth rates of B aerogenes approach the growth rate of the organism in peptone alone. In other words there seems to be no toxic, inhibitory, or stimulating effect for B aerogenes by the amino acids in the concentrations used in media containing 1% Difco Bactopeptone. Further, we may note that variation in concentrations of aspartic acid, 0.5% and 0.05%, produces no appreciable difference in growth.

In media containing these amino acids in the same concentrations, respectively, as the approximate sole source of nitrogen - approximate as the bacteria are able to grow to some extent on the base medium alone, the curves for B aerogenes show a slightly increased slope and a higher count at twenty-four hours than in the base medium. This would suggest that B aerogenes is able to utilize glycine and especially aspartic acid.

In the base medium to which 0.75% trypyophane has been

added, the curve is depressed temporarily below the initial count. However, after twenty-four hours, the count has exceeded the norm of the curve in peptone at twenty-four hours. In view of the fact that in 0.75% tryptophane-peptone medium no inhibition is produced, the explanation might be possibly that in the absence of peptone, B aerogenes of necessity adapts itself to the large tryptophane molecule and forms extracellular enzymes which digest it.

In the interpretation of the data and graph, Plate II, for B coli, we observe that the growth rates in aspartic acid-peptone medium and tryptophane-peptone medium are very similar in both low and high dilutions to the rate of growth in peptone alone. The coli organisms grow well on aspartic acid as the sole source of nitrogen. The growth of B coli communis in 0.75% tryptophane base medium suggests a possible utilization of the acid.

In 1.5% glycine base medium, the glycine is apparently utilized by B coli. However, the growth curve on the addition of 1% Difco Bactopeptone is markedly changed. Here, after the third hour, the count falls to below the initial reading. The inhibitory effect, tho, is not maintained for after 72 hours the bacterial count has risen to approximately that in peptone medium. Moreover, this effect is not produced when 0.05% glycine-1% peptone medium is used. An explanation for this increased lag period is difficult. It might be caused by a combination of glycine and some substance in the peptone neither of which

are inhibitory in themselves or in lower concentrations. The growth after the first twelve hours may have been accomplished by the adaptation of the bacteria to the inhibiting substance or substances, or the inhibiting effect may have been reduced by absorption by the organism, by spontaneous decomposition, by neutralization, or by decomposition by extracellular enzymes. It would be interesting to note whether these organisms taken after the prolonged lag period in the actively growing stage from 1.5% glycine-1% peptone medium would also have a sustained lag period when inoculated into similar sterile media. The inhibiting effects obtained by Wyom and McLeod may have been due to the added amino acid or substances present in the peptone of their media.

In examining the growth rates of B dysentery Flexner, Plate III, we find the organism multiplies slowly. No inhibitory or marked stimulating effect is apparent in peptone medium on the addition of 0.05% and 0.75% tryptophane, 1.5% glycine, or 0.5% aspartic acid. None of the amino acids in the amino acid-base media used seem to have been utilized by the Flexner bacillus. The organisms may undergo autolysis or utilize the nitrogen of nitrogenous impurities.

B paratyphosus B seems to have been unable to utilize tryptophane which also produced no inhibitory, stimulating, or toxic effect in either 0.75% or 0.05% concen-

trations. Aspartic acid seems to have been utilized. The concentration of 0.05% aspartic acid was not sufficient for growth.

Here, again, with B paratyphosus B we find the increased lag period with 1.5% glycine-1% Difco Bactopeptone as was shown with B coli. The count is even more materially decreased before the positive slope is manifested. Similarly, the lower concentration of glycine, 0.05%, in peptone does not exhibit this effect.

Recapitulating with the aid of graphs, Plates V, VI, and VII, we note that the lag phases of B paratyphosus B and B coli communis are very noticeably increased and the initial count temporarily decreased in 1.5% glycine-1% bactopeptone-base. The effect of differing concentrations of different commercial peptones on these organisms would be of interest. In 1.5% glycine base medium, B coli grows readily and the primary B paratyphosus B count is approximately sustained. B aerogenes multiplies comparatively rapidly in glycine-base medium. No stimulating, toxic, or inhibitory effect is manifested on the addition of peptone. Glycine is not used to any great extent by B dysentery Flexner.

Aspartic acid is a very favorable constituent for media for growing B aerogenes and B coli communis. The growth curve of B paratyphosus B is not greatly altered by this acid. Growth of B dysentery Flexner in peptone does not seem to be aided or hindered by theaddition of aspartic acid. The aspartic acid-base curve seems to in-

Table XXI. Changes in P_H Values of Aspartic Acid Media

Organisms	1% Peptone Base			0.5% Aspartic Acid 1% Peptone Base			0.05% Aspartic Acid 1% Peptone Base			0.5% Aspartic Acid Base			0.05% Aspartic Acid Base			Base		
	36	48	72	36	48	72	36	48	72	36	48	72	36	48	72	36	48	72
B Coli Cummunis	6.9	7.2	7.4	7.8	8.4	8.4	7.4	7.6	--	7.2	7.3	8.4	6.9	6.9	6.9	6.8	6.8	
B dysentery Flexner	6.8	6.8	6.8	6.8	6.8	7.2	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.9	6.9	6.9
B. Aerogenes	6.8	7.0	--	6.8	7.8	--	6.8	7.6	--	8.4	8.4	--	7.0	7.0	--	6.9	6.9	--

dicate that the acid is not utilized by this organism.

Altho realizing that the production of ammonia is not an indication of amino acid utilization and that the alkalinity of the media is not dependent on the production of ammonia, Ayers and Rupp (1918), the change in Ph value of the media containing aspartic acid was taken for B coli, B dysentery Flexner, and B aerogenes. These results, Table XXI, may indicate the utilization of aspartic acid by B aerogenes and B coli communis.

Tryptophane in 0.75% concentration appears to have a temporary toxic action for B aerogenes. This effect is not shown on the addition of 1% peptone. The explanation may be that tryptophane is not split when the more easily utilized peptone constituents are present. B paratyphosus B and B dysentery Flexner apparently do not utilize the acid which, further, seems to produce no effect upon the growth of the organisms with or without peptone. B coli communis is able to utilize tryptophane as the sole source of nitrogen. No visible altered effect was shown on the addition of peptone.

CONCLUSIONS

1. The failure to utilize certain simple and more complex amino acids is shown by some bacteria; as for example, B dysentery Flexner and B paratyphosus B are unable to utilize tryptophane.

2. Some organisms may show a selective utilization of amino acids. This may be illustrated by B dysentery Flexner and B paratyphosus B which utilize aspartic acid but do not, glycine and tryptophane.

3. None of the following acids, glycine in 1.5% and 0.05% concentrations, aspartic acid in 0.5% and 0.05% concentrations, and tryptophane in 0.75% and 0.05% concentrations produced any inhibiting effect when used as the sole source of nitrogen for B coli communis, B aerogenes, B dysentery Flexner, and B paratyphosus B with the exception of Tryptophane in 0.75% concentration for B aerogenes. In this case a prolonged lag period was effected which was manifested only in the tryptophane-base medium.

4. An unknown inhibiting agent, which increased the lag period for both B paratyphosus B and B coli was obtained in media containing 1.5% glycine, 1% Difco Bactopeptone, and the base medium.

5. The toxic effects in tryptophane-base media for B aerogenes, B aerogenes (61), B prodigiosus, B dysentery

Flexner, B coli communis, B paratyphosus A, B paratyphosus B, and B cloacae are manifested after the period of maximum growth and are therefore probably produced by products of decomposition.

6. No growth stimulation effect was found by any of the amino acids at concentrations used throughout the experiment.

7. Without peptone, the lag periods were prolonged and, in most cases, the growth reduced.

BIBLIOGRAPHY

Armand-Delille et al (1913) Contribution a la Biochemie des Microorganisms. Jour. de Physiol. et de Path. 1913, 15, 397.

Ayers, S. H. and Rupp, P., (1918) Simultaneous Acid and Alkaline Bacteria Fermentations from Dextrose and the Salts of Organic Acids Respectively. Jour. Inf. Dis. 1918, 23, 188.

Bainbridge, F. A., (1911) The Action of Certain Bacteria on Proteins. Jour. of Hyg. 11, 341.

Barber, M. A., (1908) Multiplication of B coli. Jour. Inf. Dis. 5, 379.

Berman, Nathan, and Rettger, L. F. Bacterial Nutrition; Further Studies on the Utilization of Protein and Non-protein-nitrogen. Jour. Bact. 1918, 3, 367-388.

Braum, H., and Cohn-Bronner, C. E., (1921) On the Synthetic Capacity Pathogenic Bacteria and their Biological Behavior in Simple Nutrients. Central bl. f. Bakteriol, I. Abt., Orig, 1921 86, 1-16.

Buchanan, R. E., (1918) Life Phases in a Bacterial Culture Jour. Inf. Dis. 23, 109-125.

Burrows, M. T., and Neymann, C. A., (1917) Studies of the Metabolism of Cells in Vitro. I. The Toxicity of the Amino Acids for Embryonic Chicken Cells. Jour. Exp. Med. 25, 93-108.

Chesney, A. M., (1916) The Latent Period in the Growth of Bacteria Jour. Exp. Med. 24, 387.

Davis and Ferry (1919) The Role of Amino Acids in the Metabolism of the Bacillus of Diphtheria. Jour. Bact. 4, 217-241.

Folin, Otto, and McCallum, A. B., (1912) On the Determination of Ammonia in Urine. Jour. Bio-chem. 11, 523-525.

Francis, E., (1923) The amino Acid Cystine in the Cultivation of B Tularense. Pub. Health Reports, Wash., 1923. 38, 1396-1404.

Franzen, H., and Egger, F., (1914) Beitrage zur Biochemie der Mikrorganismen. Ztschr. f. Physiol. Chem. 1914, 94, 311.

Galimard, J., and Lacomme, L., (1908) Les Acides amines comme nouveaux milieux de culture chemiquement definis pour l'etude des microbes. Jour. de Physiol. et Path Gen. 9, 481-496.

Gordon, J., (1924) Cystine in Bacterial Metabolism. Jour. Path. and Bact. 27, 123.

Heheworth, F. H., (1901) Arch. f. Hyg. 1901 19, 321.

Koser, S. A., and Rettger, L. F., (1919) Studies on Bacterial Nutrition Jour. Inf. Dis. 24, 301-321.

Long, E. R., (1919) A Study in Fundamentals of the Nutrition of the Tubercle Bacillus: The Utilization of Some Amino Acids and Ammonium Salts Amer. Review of Tuberculosis 1919, 3, 86.

Mayer, A., and Schaeffer, G., (1919) Role de l'Arginine et de l'Histidine dans la Culture du Bacille du Koch sur Milieux Chimiquement Definis. Condre rendu Soc. de Biol. 1919, 82, 113-115.

Muller, M., (1895) Ueber den Einfluss von Fiebertemperaturen auf die Wachsthumsgeschwindigkeit und die Virulenz des Typhus-bacillus Z. Hyg. u. Infectionskr. 1895, 24, 245.

Otsuka, Ichiro (1916) Bacterial Decomposition of Polypeptids Abs. Bact. I No. 4, 299.
Physiol. Abstr. Lond. 1917, 2, 15.

Penfold, W. J., (1914) On the Nature of Bacterial Lag. Jour. Hyg. 1914, 14, 215.

Pere, M. A., (1892) Contribution a la biologie du bacterium coli commune et du bacilli typhique. Annales de Inst. Pasteur. 6, 512-537.

Rahn, Otto, (1906) Ueber den Einfluss der Sloffinechsel Producte auf das Wachst m der Bacterien. Centralblatt f. Bakteriol 1906, 16, 417.

Raistrick, H., (1919) A Quantitive Study of the Aerobic Decomposition of Histidine by Bacteria. Bio-chem. Jour. 1919, 13, 446.

Raistrick, H., and Clark, A. B., (1920) A Quantative Study of the Aerobic Decomposition of Tryptophane and Tyrosine by Bacteria. Bio-chem. Jour. 15, 76.

Rettger, Leo F., Berman, Nathan, and Sturges, Wm. S., (1916) Further Studies on the Utilization of Protein and Non-protein Nitrogen, Jour. Bact. 1, 15-33.

Robinson, H. C., and Rettger, L. F., (1918) The growth of Bacteria in Protein-free Enzyme and Acid Digestion Products Jour. Bact. 1918, 3, 209-229.

Salter, R. C., (1919) Observations on the Rate of Growth of B coli. Jour. Inf. Dis. 1919, 24, 260-285.

Sasaki, T., (1914) Das Verhalten von Tyrosine gegen Bacteria, B coli Commune. Biochem. Ztschr. 59, 429.

Sperry, J. A., and Rettger, L. F., (1915) The Behavior of Bacteria toward Purified Animal and Vegetable Proteins. Jour. Biol. Chem. 20, 445-459.

Sturges, W. S., and Rettger, L. F., (1922) Bacterial Autolysis Jour. Bact. 7, 551-577.

Tanner, F. W., and Wallace, G. I., (1924) Relation of Temperature to the Growth of Thermophilic Bacteria. Jour. Bact. 10, 421, 1925.

Treece, E. Lee, (1926) Cultural and Metabolic Studies of the Colon-Aerogenes Group of Bacteria. Doctorate Thesis, University of Kansas.

Wagner, E., and Meyer, K. F., (1925) Studies on Metabolism of B botulinus in Various Media. Jour. Bact. 1925, 10.

Wyon, G. A., and McLeod, J. W., (1923) Preliminary Note on Inhibition of Bacterial Growth by Amino Acids. Jour. Hyg. 21, No. 4, 376-385.

Zunz, E., and Gyorgy, P. (1916) Observations sur l'Influence Chemique des Milieux de Culture sur le Developpment et la Production de l'Indole par les Colibacilles et par les Bacilles typhiques. 1916, 1, 625.